

Exhibit 1

TALCUM POWDER AND OVARIAN CANCER RISK

BY

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Note: Supporting Documents (Figure 1 and Tables 1-6 are attached separately)

BACKGROUND FOR JENNIFER BLYTHE PERMUTH, PHD, MS

I am a tenure-track Associate Professor and Molecular Epidemiologist at the H. Lee Moffitt Cancer Center and Research Institute (Moffitt; Tampa, Florida) in the Department of Cancer Epidemiology and serve as Vice Chair of Research in Gastrointestinal Oncology at Moffitt. I received a Bachelor of Science degree in Biology from the University of South Florida (Tampa), a Master of Science degree in Molecular, Cellular, and Developmental Biology and Genetics with specialization in Genetic Counseling from the University of Minnesota (Minneapolis/St. Paul, MN), and a doctorate in Epidemiology from the University of South Florida College of Public Health. During my career as a genetic counselor, I personally counseled and educated hundreds of women newly diagnosed with ovarian cancer and related cancers (primary peritoneal and fallopian tube cancer) about possible causes of their disease, along with implications for their family members. I have also been an active investigator involved in studying the genetic epidemiology of ovarian cancer as part of the Ovarian Cancer Association Consortium (OCAC), an international collaboration comprised of investigators who are conducting epidemiological studies on ovarian cancer. I am also known for my research and patient advocacy work related to another lethal malignancy, pancreatic cancer. I have a very active research portfolio, and am currently funded with grants from numerous health agencies, including an R01/R37 grant from the National Cancer Institute (NCI) and a Translational Research Partnership grant from the Department of Defense. I have authored more than 100 peer-reviewed papers, reviews, book chapters, and other reports in the areas of genetics, epidemiology, molecular biology, and gynecologic and gastrointestinal oncology. These publications include several review articles and a book chapter regarding the epidemiology of ovarian cancer, as well as several meta-analyses (see Curriculum Vitae, attached as exhibit A). I also serve as a peer reviewer of articles in these areas for top-tier journals and as a grant reviewer at the NCI.

In this report, I address causes of and risk factors for ovarian cancer and will challenge the fundamental bases of plaintiffs' experts' hypotheses and contentions regarding talc as a causal agent. The opinions in this report are my own and do not necessarily represent opinions of my institution or the agencies by which I am funded. My

hourly rate for serving as an expert is \$750. I have served as an expert in the talc litigation and have been deposed twice. I have testified once as an expert witness in a talc trial.

SUMMARY OF THE ORGANIZATION OF THIS REPORT

This report is organized into seven main sections. Section I includes an overview regarding ovarian cancer (OvCa) and contains pertinent statistics, information about its pathologic classification, and descriptive epidemiology. Section I also includes a comprehensive review of scientific literature, updated from my prior publications, regarding established and probable risk and preventive factors for OvCa, along with factors for which there is inconclusive or insufficient evidence to support causality.¹⁻⁴ Section II includes an overview of the methodology and analyses that have been used to evaluate the posited association between talc and OvCa. This framework is intended to serve as a foundation for concepts and opinions presented in subsequent sections of this report. Section II begins with an overview of the different types of epidemiologic study designs, along with their various strengths and limitations. A comprehensive review of the epidemiologic studies that have evaluated the reported association between talc and OvCa follows, with individual studies discussed first, followed by pooled and meta-analyses. Section II concludes with a summary of laboratory-based studies of talc that have been conducted in animals, in vitro, or using human tissue. Section III provides an overview of guidelines for assessing causation and highlights the importance of integrating data from disciplines in addition to epidemiology when evaluating the hypothesized perineal talc-OvCa association. Section III concludes with a summary of methodological, statistical, and biological concerns regarding the talc literature to date. Section IV summarizes reports from national and international agencies that study substances/agents to determine whether they cause cancer. In section V and throughout earlier sections of this report, commentary is provided on the reports submitted by plaintiffs' epidemiology experts. An overall summary and final conclusions are presented in Section VI, followed by a list of cited references in Section VII. Supporting documents (Figure 1 and Tables 1-6) are appended as Exhibits B (Figure 1) and C (Tables 1-6). Based on the totality of available evidence, it is my opinion to a reasonable degree of scientific certainty that the plaintiffs' experts' hypotheses as to causation are flawed.

Reliable and robust scientific evidence is *lacking* to support the contention that talc causes OvCa.

I. OVERVIEW OF OVARIAN CANCER (OvCa)

A. Incidence and mortality rates

In 2024, approximately 19,680 new cases of OvCa and 12,740 OvCa related deaths are expected to occur in the US.⁵ OvCa accounts for 5% of cancer deaths among females, which is more than any other cancer of the female reproductive system. The average lifetime risk of an American woman developing OvCa is 1 in 87 (1.14%), and her chance of dying of the disease is 1 in 130 (0.76%).⁵ The median age of diagnosis for OvCa is 63 years.⁵ The disease typically presents at late stage, when the 5-year relative survival rate is only 31%; few cases (17%) are diagnosed with localized tumor (stage 1) when the 5-year survival rate is 93%.⁵ In the US, rates for new OvCa cases have been falling on average 3.2% each year from 2010-2019, and death rates have been falling on average 2.8% each year from 2011-2020.⁶

B. Pathologic classification

The ovaries are female reproductive glands located on each side of the uterus that serve two main roles: 1) to produce eggs, which are fertilized during reproduction; and 2) to function as the primary source of the hormones estrogen and progesterone among pre-menopausal women.⁵ Tumors can develop when cells grow uncontrollably. Nearly all benign and malignant ovarian tumors originate from one of three cell types: epithelial cells, stromal cells, and germ cells. In developed countries, more than 90% of malignant ovarian tumors are epithelial in origin, 3% are germ cell tumors (e.g., teratomas, dysgerminomas, etc.), and approximately 2% of tumors constitute sex cord-stromal tumors (e.g., granulosa cell tumors, thecomas, etc.).⁵ The age distribution of OvCa varies by tumor type, with epithelial tumors occurring primarily in the late 70s, sex cord stromal tumors in the 50s, and germ cell tumors around ages 15-19.⁵ Most epidemiologic research, including the present report, focuses on epithelial OvCa.

Epithelial OvCa reflects a heterogeneous disease with histologic subtypes (histotypes) that differ in their cellular origin, risk factors, pathogenesis, molecular alterations, gene expression and prognosis.⁷⁻¹¹ Malignant OvCa, also known as carcinomas, are comprised of five main histotypes: high-grade serous (HGSOC; 52-70%),

endometrioid (ENOC; 10%), clear cell (CCOC; 6-10%), mucinous (MOC; 3-6%) and low-grade serous (LGSOC; <5%).^{7,8,12} Within each of these categories are tumors of uncertain malignant behavior, known as borderline or low malignant potential (LMP) tumors; they contain microscopic features of malignancy without frank invasion into surrounding stroma.¹³ As such, women with these borderline tumors can have five-year relative survival rates greater than 98%.¹⁴

The cellular origin and pathogenesis of OvCa are not well understood. However, we now know that most OvCa tumors appear to originate from other gynecological tissues, such as fallopian tubes, and involve the ovary secondarily. Moreover, epithelial subtypes are increasingly characterized as distinct diseases with different molecular pathways (and possibly risk factors), as will be mentioned throughout this report and summarized near the end in a table. Morphological and genetic studies have given rise to several hypotheses of origination, particularly for HGSOC, which is characterized by involvement of both ovaries, aggressive behavior, late-stage diagnosis and poor survival.^{15,16} Compelling data suggest high- and low-grade serous neoplasms originate from fallopian tube epithelium, CCOC and ENOC from the endometrium (lining of the uterus), and MOC from transitional cell nests at the tubal-mesothelial junction.^{17,18} HGSOC and LGSOC are both believed to arise from tubal epithelium, but through separate pathways. Atypical lesions within the fimbriated end of the fallopian tube (serous tubal intraepithelial carcinomas [STIC]) resemble HGSOC tumors because of their similar morphology and molecular profiles, suggesting the neoplastic process may originate at these tubal lesions and spread to the ovary, where they progress.¹⁹⁻²¹ LGSOC tumors present along a continuum that clearly progresses from benign serous cystadenoma to borderline serous tumor and then low-grade carcinoma. Similar to low-grade serous tumors, mucinous, endometrioid, and clear cell carcinomas are thought to progress from borderline tumors in a stepwise manner and are designated as Type I tumors.²² Type I and Type II tumors display different, often mutually exclusive, mutational profiles. Type I tumors are associated with mutations in *BRAF* and *KRAS* oncogenes in LGSOC and mucinous tumors, and *PTEN* in endometrioid tumors, none of which is characteristic of HGSOC tumors, of which nearly 100% have p53 mutations.^{22,23} Moreover, some risk and preventive factors vary by the major histotypes.^{24-32,33} For example, tubal ligation, two or

more births, endometriosis and age have been reported to be more strongly associated with mucinous, endometrioid and clear cell tumors than serous tumors.³³

In a study conducted by the Ovarian Cancer Cohort Consortium (OC3),³² associations between 14 hormonal, reproductive and lifestyle factors and OvCa risk were evaluated by histologic subtype in a sample of 5,584 invasive epithelial OvCa cases (3,378 serous, 606 endometrioid, 331 mucinous, 269 clear cell and 1,000 other). Higher parity was most strongly associated with endometrioid (relative risk [RR] per birth, 0.78, 95% CI, 0.74 to 0.83) and clear cell (RR, 0.68, 95% CI, 0.61 to 0.76) carcinomas. Similarly, age at menopause, endometriosis and tubal ligation were only associated with endometrioid and clear cell tumors (P-het \leq .01). Smoking was associated with an increased risk of mucinous (RR per 20 pack-years, 1.26, 95% CI, 1.08 to 1.46) but a decreased risk of clear cell (RR, 0.72, 95% CI, 0.55 to 0.94) tumors (P-het = .004). Most established risk factors were more strongly associated with non-serous carcinomas, which demonstrate challenges for risk prediction of serous cancers, the most fatal subtype.

Of note, primary peritoneal cancer and fallopian tube cancer of serous type are rare malignancies that share similarities in clinical presentation and management with HGSOC.³⁴ However, a critical review of the literature conducted by Sorensen et al.³⁵ suggests primary peritoneal cancers differ from OvCa and fallopian tube cancers in their risk factor profile, molecular patterns and prognosis. For example, compared to OvCa patients, patients with primary peritoneal cancer tended to be older, have higher parity, were more often obese and had poorer survival. Primary peritoneal cancers may also have a more aggressive biology characterized by increased Ki67 and differences in protein expression of Her2/neu, estrogen and progesterin receptors, and the frequency of loss of heterozygosity, suggesting that primary peritoneal and OvCa may be linked to different carcinogenic pathways.³⁵ Thus, it is important to make a distinction regarding the primary site of origin. Caution should be taken when trying to extrapolate findings from studies based on women with OvCa to those of women with primary peritoneal carcinoma.

C. Descriptive Epidemiology of OvCa

The highest age-adjusted incidence rates of OvCa are observed in developed parts of the world, including North America and Central and Eastern Europe, with rates more than 8 per 100,000. Rates are intermediate in South America (5.8 per 100,000), and lowest in Asia and Africa (≤ 3 per 100,000). Migration from countries with low rates to those with high rates results in greater risk,^{36,37} underscoring the importance of non-genetic risk factors. Additionally, limited access to high quality care and lack of guideline adherent care may contribute to increased risk and poorer outcomes.³⁸ Within the US, racial differences in incidence and mortality mimic the observed international variation, with rates highest among Non-Hispanic Whites (NHW), intermediate for Hispanics, and lowest among Blacks and Asians.³⁹ It has been suggested that part of the variation in OvCa risk may be attributed to differences in the prevalence of reproductive factors including the number of childbirths, oral contraceptive use, and tubal ligation, however the origin of most of the variation is yet to be determined.⁴⁰ During 2004-2014, overall OvCa incidence rates in NHW were 30% higher than those in Non-Hispanic Blacks and Asian American/Pacific Islander women. However, Black women are reported to have the second highest mortality rates, which may be explained by a more advanced stage at diagnosis, a lower probability of receiving standard-of-care treatment such as surgery followed by chemotherapy, and various comorbidities.^{41,42} However, Bristow et al.⁴² showed that adherence to treatment guidelines for advanced-stage OvCa is associated with equivalent survival benefit across racial or ethnic and socioeconomic strata. Thus, ensuring equal access to standard treatment is a viable strategic approach to reduce survival disparities.

D. Risk And Preventive Factors

i. Established or probable factors.

Family history and genetic predisposition

The strongest risk factor for OvCa is having a family history of or genetic predisposition to OvCa or breast cancer.^{43,44} First-degree relatives of OvCa cases have a 3- to 7-fold increased risk, especially if multiple relatives are affected, and at an early age of onset.⁴⁵⁻⁴⁹ Risk is increased by approximately 70% among women with a history of breast cancer.⁵⁰ Up to 20% of OvCa cases are due to rare, high penetrant mutations

in genes such as *BRCA1* and *BRCA2*.⁵¹⁻⁶⁰ Data from the Breast Cancer Linkage Consortium suggest the risk of OvCa through age 70 years is up to 44% in *BRCA1* families⁶¹ and approaches 27% in *BRCA2* families.⁶² The risk of developing OvCa by age 80 is 44% and 17% in *BRCA1* and *BRCA2* mutation carriers, respectively.⁶³ Most OvCa cases associated with *BRCA1/2* mutations have high-grade serous histology, but non-serous histologies such as clear cell and endometrioid subtypes have also been reported among *BRCA1/2* mutation carriers.^{54,64} Although *BRCA1/2* mutations are rare (<1% in the general population), they occur more frequently in certain ethnic or geographically isolated populations, such as those of Ashkenazi Jewish (Central European) descent, where the prevalence is ~2.5%.⁶⁵ Hereditary Non-Polyposis Colorectal Cancer Syndrome (HNPCC)⁶⁶ may account for at least 2% of OvCa and confer an 8-20% lifetime risk.^{51,67-}

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As summarized recently by Johansen et al.⁷³ and Kostov et al.⁷⁴, since the time the *BRCA1/2* genes were discovered, OvCa risk (for serous and non-serous histologies) has been associated with mutations in approximately 20 additional genes including *MLH1*, *MSH2*, *MSH6*, *PMS2*, *MUTYH*, *EPCAM*, *TP53*, *CDH1*, *MRE11*, *ATM*, *RAD50*, *BARD1*, *PTEN*, *RAD51C*, *RAD51D*, *NBN*, *CHEK2*, *BRIP1*, and *PALB2*. Other authors have similarly reported that cases of epithelial OvCa have been reported in families with *TP53* mutations and Li Fraumeni Syndrome and in families with *PTEN* mutations and Cowden Syndrome; those with Cowden syndrome tend to be of endometrioid histology though other histologies have been reported.^{75,76} Additionally, although mutations in the *MUTYH* gene are most commonly associated with polyposis and colorectal cancer, Win and colleagues⁷⁷ have reported a 14% increased risk for OvCa risk among biallelic *MUTYH* mutation carriers, with serous carcinomas being the most common histology identified. As described by Hutchcraft et al.⁷⁸, a monoallelic germline mutation combined with a somatic *MUTYH* mutation may also contribute to OvCa development and therapeutic response, and monoallelic *MUTYH* mutations have been reported among women with ovarian and/or breast cancer.⁷⁹ Women with mutations in DNA repair genes, such as *BRIP1*, *RAD51C*, and *RAD51D* have estimated OvCa lifetime risks of 5.8%, 5.2% and 12%, respectively.^{80,81} Germline mutations in the Fanconi anemia genes (e.g., *FANCC*) have also been reported in women with OvCa and/or breast cancer.⁸²⁻⁸⁷

Deleterious mutations in *BRCA1/2* and other double-strand DNA break repair genes are more strongly associated with HGSOC susceptibility, although these mutations do occur in other tumor subtypes.^{80,81,88} HNPCC-associated OvCa typically presents as endometrioid or clear cell tumors, rather than the common serous subtype.^{89,90} For all these reasons, the National Comprehensive Cancer Network recommends germline genetic testing for all women affected by epithelial OvCa.⁹¹ Tumor genomic sequencing is also recommended for all women with epithelial OvCa;⁹¹ this approach can serve as a complement to germline genetic testing and may also identify heritable mutations and phenomena including constitutional low-level germline mosaicism.^{64,92-95}

Collectively, known syndromes account for 25-36% of OvCa familial relative risk.^{96,97} Genome-wide association studies (GWAS)⁹⁸⁻¹⁰⁸ have discovered more than 20 susceptibility alleles for invasive OvCa with weak to moderate effects in European populations. Eighteen of these risk loci are associated with OvCa overall and/or serous OvCa, five are associated with MOC risk, one is associated with ENOC, and one is associated with CCOC, exemplifying the genetic heterogeneity of OvCa by histotype. The identified common risk alleles account for approximately 4% of the polygenic risk of OvCa in the European population and, taken together with high risk alleles, explain 40% of the heritability.¹⁰⁹ A recent GWAS undertaken to identify EOC loci in women of African ancestry also identified 10 novel loci and reported shared genetic architecture between women of African and European populations.¹¹⁰ Genetic variants interrogated in GWAS have also been used to evaluate associations with established and possible OvCa risk factors.¹¹¹ To date, findings from GWAS of OvCa have not been translated to the clinical setting to aid in risk prediction.

Hormonal and Reproductive Factors

Epidemiological research has consistently implicated hormonal and reproductive factors in the pathogenesis of OvCa. Several predominant hypotheses have emerged to fit the data.¹¹² The “incessant ovulation” hypothesis posits that the number of ovulatory cycles increases the rate of cellular division associated with the repair of the surface epithelium after each ovulation, thereby increasing spontaneous mutations that can in turn lead to pre-malignant or malignant phenotypes.¹¹³ The correlation between increasing numbers of lifetime ovulatory cycles (LOC) and higher OvCa risk aligns with

this hypothesis, especially for tumors of high-grade serous and other non-mucinous histologies.¹¹⁴⁻¹²⁵ For example, the relationship between LOC and OvCa risk was evaluated by Trabert and the Ovarian Cancer Cohort Consortium (OC3) in an analysis of 20 prospective cohorts and over 558,000 post-menopausal women among whom 3,246 developed invasive EOC (including 2,045 serous, 319 endometrioid, 184 mucinous, and 121 clear cell).¹²⁵ Compared to women in the lowest LOC percentile (<294 cycles), women in the highest LOC percentile (>514 cycles) were nearly two times more likely to be diagnosed with OvCa (<294) (HR (95% CI: 1.92 (1.60-2.30)). OvCa risk increased 14% (95% CI: 1.10-1.17) for each 5-year increase in LOC (60 cycles); risk remained after adjustment for parity and oral contraceptive use [1.08 (1.04-1.12)]. The association varied by histotype, with increased risk of serous [1.13 (1.09-1.17)], endometrioid [1.20 (1.10-1.32)], and clear cell [1.37 (1.18-1.58)], but not mucinous [0.99 (0.88-1.10), P-heterogeneity = 0.01] tumors.¹²⁵ The relationship between lifetime ovulatory years (LOY) and EOC risk was assessed in 2022 via a systematic review and meta-analysis of 24 studies.¹¹⁹ Compared to women with the lowest level of LOY, women with the highest level of LOY were found to have >2.26 times higher odds of EOC (95% CI 1.94-2.83). Associations were observed between LOY and serous (pooled OR 2.31, 95% CI 1.60-3.33) and endometrioid histology (pooled OR 3.05, 95% CI 2.08-4.45) but not mucinous histology (pooled OR 1.52, 95% CI 0.87-2.64). In 2023, Fu et al.¹¹⁸ published a pooled analysis on the association between LOY and EOC risk among 21,267 cases and 26,204 controls from 25 studies participating in the Ovarian Cancer Association Consortium, with many studies overlapping with the prior meta-analysis.¹¹⁹ They¹¹⁸ showed that LOY was associated with high-grade serous, low-grade serous, endometrioid, and clear cell histotypes, with increases in OR per year of 1.05, 1.04, 1.07, and 1.1, respectively; no increases were observed for mucinous tumors. Finally, also in 2023, Cramer et al.¹²⁶ evaluated sex hormone levels in post-menopausal women from the Nurses' Health Study and showed that higher testosterone levels are associated with greater LOY and that higher estradiol was detected among those with increased BMI. They¹²⁶ concluded that repeated ovulations and conversion of androgens such as testosterone may explain why greater LOY may increase the risk for OvCa, breast cancer, and endometrial cancer.

Taken together, having high LOY significantly increases the risk for OvCa, in support of the incessant ovulation hypothesis.

The “gonadotropin hypothesis” posits that stimulation of the ovarian surface epithelium by gonadotropins (luteinizing hormone and follicle-stimulating hormone) promotes an increased OvCa risk by overstimulating the ovarian epithelium, causing increased proliferation and subsequent malignant transformation.¹²⁷ The close temporal relationship between a rise in gonadotropin levels and increased OvCa incidence during menopause supports this theory.¹²⁸ Risch¹¹² has also hypothesized that OvCa risk may be increased by factors associated with a strong androgenic stimulation of ovarian epithelial cells and decreased by factors related to increased progesterone stimulation. In support of this theory are animal models that show a high concentration of androgens inside the ovarian stroma surrounding epithelial inclusion cysts,^{129,130} along with epidemiologic studies showing elevations of androgens in women with or at high risk for OvCa.^{131,132} However, some studies have refuted this hypothesis.^{133,134} A more recent study¹³⁵ conducted by the European Prospective Investigation of Cancer and Nutrition (EPIC) cohort reported a positive (but not statistically significant) association for low-grade (OR= 1.99 [0.98-4.06]) and decreased or protective associations (i.e., OR<1.0) for high-grade carcinomas (OR= 0.75 [0.61-0.93]) for both type I/II tumors. These proposed mechanisms provide a framework to interpret the epidemiologic data on both endogenous correlates of reproductive hormone exposure and exogenous sources of hormones.

According to the incessant ovulation hypothesis, early age at menarche and late age at menopause increase risk by increasing the number of ovulatory cycles. Conversely, according to the gonadotropin hypothesis, a late age at menopause delays the surge of post-menopausal gonadotropin hormones, possibly reducing risk. Results of studies that have examined the age at onset of menses are inconsistent,^{132,136-145} though a more recent meta-analysis yields an overall inverse association between age at menarche and OvCa risk (RR=0.85, 95% CI: 0.75-0.97).¹⁴⁶ Data on age at natural menopause and OvCa risk are also inconsistent. Case-control studies have reported odds ratios ranging from 1.4 to 4.6 in the highest category of age at menopause.^{136,137,139,143,147-149} In the EPIC cohort, age at menopause (>52 versus ≤45 years) was associated with an increased OvCa risk (HR= 1.57, 95% CI: 1.16–2.13);

however, after women diagnosed with OvCa within the first two years of follow-up were excluded, the risk was slightly attenuated and not statistically significant (HR=1.40, 95% CI: 0.98-2.00).¹⁵⁰ The authors speculated that post-menopausal women in the sub-clinical stage of OvCa may mistake bleeding for menses. Other case-control studies^{132,142,151-155} and several cohort studies^{144,156} found no association. The inconsistent findings between ages at menarche and menopause and OvCa risk may reflect differences in definitions, recall and misclassification bias or differences in analysis.¹⁵⁷ The etiologic heterogeneity of tumor subtypes may also contribute to differential findings. A report from the Nurses' Health Study (NHS) and Nurses' Health Study II found that age at natural menopause was associated with an increased risk of endometrioid tumors (RR= 1.13, 95% CI: 1.04–1.22), but not serous invasive or mucinous tumors.³¹ Studies conducted among populations with different distributions of age at menarche^{143,158,159} and age at menopause¹⁶⁰ indicate differences in the heritability of factors across ancestral groups.¹⁶¹⁻¹⁶³ Nevertheless, evidence suggests any magnitude of effect is likely small for age at menarche or age at menopause by themselves and that lifetime ovulatory history should be considered.^{118,119,125}

Parity

The association between pregnancy and OvCa risk has been studied extensively. Pregnancy causes anovulation and suppresses secretion of pituitary gonadotropins, and is thus consistent with both the 'incessant ovulation' and the 'gonadotropin' hypotheses. Indeed, parous women have a 30-60% lower risk for OvCa than nulliparous women,^{113,136,143,148,149,152,154-157,164-167} and each additional full-term pregnancy lowers risk by approximately 15%.^{142,156,168} Studies in Black¹⁶⁹ and Asian^{170,171} populations have yielded similar results. The protective effect associated with parity is evident across OvCa histotypes, but is weaker for serous carcinomas, with ~20% lower risk in nulliparous women versus other subtypes, particularly clear cell and endometrioid, which show 50-70% reductions in risk.^{30-32,172,173} Recent data also suggest that OvCa risk does not vary by the time interval between the first and last birth.¹⁷⁴

It is unclear whether spontaneous or induced abortions impact OvCa risk. Numerous studies found that an increased number of incomplete pregnancies may slightly decrease risk,^{113,136,141,142,149,156,175-177} while others have reported risk to be

increased^{155,167} or not affected.^{132,140,143,145,152,154,164,166,178} Induced abortions have been associated with lower risk in several studies,^{156,176,177} but not others.^{140,147,175} With regard to spontaneous abortions, positive,^{132,164,175} inverse,¹⁴⁵ and null associations^{148,166,176} with OvCa risk have been reported. Interpretation of this literature is difficult because of the recognized potential for recall bias of spontaneous or induced pregnancies.¹⁷⁹

Fertility

Infertility refers to a heterogeneous group of biologically distinct conditions ranging from genital tract infections and tubal disturbances to medical conditions such as endometriosis and polycystic ovarian syndrome.^{180,181} Infertility appears to be a risk factor in most studies,^{136,142,145,152,154,164,166,167,180,182} but not all.^{156,183} The inconsistent results may reflect the failure to examine the various types of infertility separately. It is yet to be determined whether nulliparity and low parity *per se*, rather than difficulty becoming pregnant due to female infertility, is the relevant factor. Infertility appears to pose the greatest risk among women who remain nulliparous, while periods of temporary infertility among parous women are of little concern.^{136,142,145,154,166} For example, in a large Canadian case-control study in which most nulliparous women were so by choice, infertility was not associated with OvCa risk among parous women, but there was a trend towards elevated risk among a small group of infertile nulliparous women (OR=2.5, 95% CI: 0.6-4.1).¹⁴⁵ One challenge is distinguishing an influence of infertility from an adverse effect of fertility drug exposure. Although some studies report that women with a prior history of fertility drug use who remain nulliparous are at an elevated risk for ovarian tumors, particularly tumors of low malignant potential,^{142,184} the results are not consistent.^{180,181,183,185-187} Early detection bias may explain the discrepant findings, as early-stage cancers may be over-diagnosed in infertile women due to close medical surveillance.¹⁸⁸ To complicate matters, some factors may influence both infertility and OvCa risk, such as a personal history of endometriosis,¹⁸⁹⁻¹⁹¹ polycystic ovarian syndrome¹⁹² and *BRCA1* mutations.¹⁹³

Lactation

Breastfeeding, or lactation, suppresses secretion of pituitary gonadotropins and leads to anovulation, particularly in the initial months after delivery.¹⁹⁴ Both the incessant ovulation and gonadotropin hypotheses would predict that lactation reduces the risk of

OvCa. Indeed, most studies show a slight protective effect from breastfeeding, with odds ratios approximating 0.6-0.7,^{142,143,145,165-167,195-198} while others have not.^{132,140,152} Few studies have explored the association by tumor subtype, with one report of the greatest risk reduction for endometrioid tumors,¹⁹⁹ while another observed the strongest reduction among mucinous cancers.³² A meta-analysis from 2014 indicates a significant protective effect (summary RR= 0.68, 95% CI: 0.61-0.76) for breastfeeding that increased with longer duration (summary RR=0.85, 0.73, and 0.64 for <6 months, 6-12 months, and >12 months of total breastfeeding duration).²⁰⁰ A recent pooled analysis²⁰¹ was performed to evaluate the association between breastfeeding and OvCa overall and by histologic subtype among parous women with EOC (n= 9,973) and 13,843 controls from 13 case-control studies participating in the Ovarian Cancer Association Consortium. Breastfeeding was associated with a 24% reduced risk of invasive EOC (odds ratio [OR], 0.76, 95% CI, 0.71-0.80), especially high-grade serous and endometrioid subtypes. Mean breastfeeding duration of 1-3 months was associated with nearly a 20% OvCa lower risk (OR, 0.82, 95% CI, 0.76-0.88), while breastfeeding for 1 year or longer was associated with a 34% lower risk (OR, 0.66, 95% CI, 0.58-0.75); the reduced risk lasted for three decades (OR, 0.83, 95% CI, 0.77-0.90; P trend = .02). Taken together, lactation is a modifiable factor that protects against EOC especially for long-term duration. Experts from the National Center Institute Screening and Prevention board have listed breastfeeding as a factor with adequate evidence of a decreased risk for OvCa.²⁰²

Oral contraceptives (OC) and other forms of contraception

Epidemiologic literature has consistently reported that use of oral contraceptives is inversely associated with OvCa risk. The protective effect increases with longer duration of use,^{142,145,203-207} with about a 20-50% decreased risk that persists decades after use has ceased.^{152,165,205,208-213} For example, a large study of 23,257 women with OvCa and 87,303 controls²¹² showed that among women who use OCs for five to nine years total, risk decreased by approximately 35%, and the protective effect remained for at least 10 years after OC use was discontinued.^{212,214} Moreover, the risk reduction did not appear to be specific to any particular oral contraceptive formulation^{207,215} or OvCa histotype, although OC use appears less effective for mucinous cancers in some studies.^{25,29,30,32,158,172,212} Oral contraceptive use corresponds to the prevention of

approximately 30,000 OvCa cases every year and has already prevented approximately 200,000 OvCa cases and 100,000 deaths over the last 50 years.²¹² Progestin-only contraceptives have been less studied, mostly due to the low prevalence of use, but the available data suggest they may also lower OvCa risk.^{165,205,216} In fact, a recent large prospective population-based study of women aged 15-49 in Denmark revealed that current or recent use of combined OC containing newer contemporary formulations of progestogen is associated with a reduced OvCa risk.²¹³ The risk reduction was similar among the serous, endometrioid and mucinous subtypes of OvCa, and the effect strengthened with longer periods of use and persisted for up to 10 years after stopping use. In contrast, progestogen-only products did not appear to protect against OvCa in this cohort, which contrasts with findings from a study in Finland,²¹⁷ possibly because the Finnish study did not adjust for parity or previous OC use, factors known to have a persistent protective effect.

Few studies have examined methods of contraception other than OCs. The use of an intrauterine device (IUD) has been associated with reduced OvCa risk in several studies,²¹⁷⁻²¹⁹ while the Nurses' Health Study cohort observed increased risks;²¹⁴ however, there was a low prevalence of IUD use in the NHS population, which occurred prior to the newer IUD formulations. Similar to OC, any protective effect associated with IUD use may be dependent upon duration of use. Several recent systematic reviews and meta-analyses have been performed to evaluate the association between IUD use and OvCa risk, with each showing an approximate 30% OvCa risk reduction with ever-use of an IUD.^{220,221} The most recent meta-analysis involved 11 studies and found that IUD use was associated with an OR=0.68 (95% CI: 0.62-0.75).²²¹ Of note, the risk of pelvic inflammatory disease (PID) attributed to IUDs has been reported to be lower than 1%.²²² Vasectomy has been evaluated in association with OvCa risk and findings have been inconclusive,²¹⁴ although Ness and colleagues²¹⁹ reported that vasectomy may confer a small reduction in risk (adjusted OR=0.77, 95% CI: 0.61-0.99), perhaps due to reduced exposure to sperm. Given that contraceptive methods are modifiable, further research to replicate these findings is needed.

Hormone Replacement Therapy (HRT)

Unlike oral contraceptive use, which has a well-established benefit against OvCa risk, the association with HRT is less clear. HRT reduces the secretion of gonadotropins and should therefore decrease risk (if the gonadotropin hypothesis is valid).²²³ Conversely, post-menopausal HRT may enhance estrogen-induced proliferation of ovarian cells, and therefore increase risk.²²⁴ Initial studies on the topic have focused on unopposed estrogen therapy among post-menopausal women. Several case-control,^{142,225,226} cohort,²²⁷ and meta-analysis^{228,229} studies have found no association with duration of use, although two have observed either a significant or suggestive trend in increased risk.^{25,230} More recent studies indicate that OvCa risk is increased in ever users of HRT,²³¹⁻²³⁴ and larger increases are seen for longer durations of HRT use.²³⁵⁻²³⁹ For example, in the Nurses' Health Study cohort, both current and past HRT users of five or more years had a significantly higher risk for OvCa than never users (RR=1.41, 95% CI: 1.07-1.86 and RR=1.52, 95% CI: 1.01-2.27, respectively), but no association with OvCa risk was seen for HRT users of less than five years for either current or past users (RR=1.01, 95% CI: 0.70-1.44 and RR=0.88, 95% CI: 0.64-1.19, respectively).²³⁵ The authors concluded that the elevated risk appeared to be driven largely by duration rather than by status of use. Conversely, a collaborative re-analysis of 52 epidemiological studies found that OvCa risk was increased in current HRT users, even those with less than 5 years of use (RR 1.43, 95% CI 1.31-1.56; $p < 0.0001$).²⁴⁰ Furthermore, OvCa risk decreased over time after cessation of use, although a small excess in serous and endometrioid OvCa risk was still observed even 10 years after stopping long-duration HRT. The authors²⁴⁰ concluded that "the increased risk may well be largely or wholly causal; if it is, women who use hormone therapy for 5 years from around age 50 years have about one extra ovarian cancer per 1000 users and, if its prognosis is typical, about one extra ovarian cancer death per 1,700 users."

Combined estrogen and progestin use and OvCa risk have only recently been evaluated in studies with sufficient statistical power. It has been hypothesized that progestins promote apoptosis while estrogen promotes proliferation of ovarian epithelial cells;²⁴¹ thus, the effects of unopposed estrogen therapy (ET) are thought to be more detrimental to the ovaries than estrogen plus progestin (EPT).²⁴¹ Most studies that

investigated EPT use and OvCa risk have found no association or a weak protective association.^{158,231,232,234,235,238,241-243} A few prospective studies^{231,237,244} and a meta-analysis²³³ have reported a small increased risk for EPT users compared to ET only users. For example, a recent meta-analysis of 14 population-based studies concluded that ET is associated with a 22% increased risk of OvCa per 5-year increment of use; however, the risk among women who used EPT was attenuated to only a 10% increase.²³² The authors suggest that the addition of progestin mitigates the effect of estrogen, because the increased risk of OvCa among EPT users was statistically significantly lower than the risk among ET users ($p = 0.004$).²³² However, several prospective cohort studies observed similar increased risks for both ET users and EPT users.^{244,245} The basis for the inconsistent literature is not readily apparent. In 2023, Lee et al.²⁴⁶ aimed to investigate the impact of HRT on OvCa occurrence in post-menopausal women with a history of endometriosis among 10,304 women that received HRT and a control group of 10,304 that did not. An increased OvCa risk was only found among ET-only HRT users (HR 2.89, 95% CI 1.25-6.72; $p = 0.013$) in this cohort of post-menopausal women with a history of endometriosis.

Some studies have indicated that any HRT-associated risk is limited to specific histologic subtypes. In the Nurses' Health Study, the increased risk was slightly stronger for endometrioid tumors and was not present for mucinous tumors, consistent with other studies.^{31,32,172,226,247} Endometrioid tumors are histologically similar to endometrial tissue,²⁴⁸ and ET use increases the risk of endometrial cancer,²²⁴ enhancing plausibility.

Obesity

In post-menopausal women, the predominant source of circulating estrogens is aromatization of androgens in adipose tissue.^{112,249} The compelling role of obesity in the pathogenesis of hormone-related cancers, such as endometrial and post-menopausal breast cancers,²⁵⁰ has prompted research on the potential association with OvCa.²⁵¹ One measure of interest is body mass index (BMI), calculated as weight in kilograms divided by height in meters squared. A 2007 meta-analysis of 28 population studies reported an increased risk of OvCa for overweight women (BMI of 25-29.9 kg/m²) and obese women (BMI ≥ 30 kg/m²), compared with normal weight (BMI of 18.5-24.9 kg/m²), pooled RR=1.2 and 1.3, respectively.²⁵² In a 2008 analysis of 12 prospective cohort studies, an increased

OvCa risk was seen among pre-menopausal obese women compared to normal weight women (RR= 1.72, 95% CI: 1.02-2.89); however, this increased risk was not apparent among post-menopausal women (RR= 1.07, 95% CI: 0.87-1.33).²⁵³ A more recent analysis of 12 case-control studies by the Ovarian Cancer Association Consortium (OCAC) also found that the positive association with BMI was stronger among pre-menopausal women.²⁵⁴ Conversely, the EPIC cohort study observed the strongest risk associations for BMI and weight among post-menopausal women.²⁵⁵ In the Nurses' Health Study (NHS), greater hip circumference, a measure of fat distribution, was a risk factor among post-menopausal women, but waist-to-hip ratio (WHR), waist circumference and BMI were not.²⁵⁶

Several studies have evaluated obesity and OvCa risk stratified by HRT use.²⁵⁴⁻²⁵⁹ The results for BMI did not differ by HRT use in the OCAC analysis, NHS or EPIC study. In contrast, three studies observed an increased OvCa risk only for obese women who have never used HRT ((RR 1.8 (95% CI: 1.2-2.8)²⁵⁷ and RR=1.10 (95% CI: 1.07-1.13))²⁵⁹ and for never-HRT-users with greater weight gain since age 18 (RR= 1.8, 95% CI: 1.0-3.0 for ≥ 40 lbs. vs stable weight), a larger waist circumference (RR= 1.8, 95% CI: 1.1.-3.0 for ≥ 35 vs < 35 inches) and a larger waist-to-height ratio (RR= 1.8, 95% CI: 1.1.-3.1 for ≥ 35 vs < 35 inches).²⁵⁸

The risk associated with obesity may be specific to non-serous and low-grade serous subtypes. Two large-scale pooled analyses, one performed by OCAC²⁵⁴ and another by the Collaborative Group on Epidemiological Studies of Ovarian Cancer,²⁵⁹ observed the strongest risk increases for borderline serous tumors (OR/RR=1.24 and 1.29 per 5 kg/m², respectively) and somewhat lower increases for clear cell (OR/RR=1.06 and 1.05 per 5 kg/m²), mucinous (OR/RR=1.19 and 1.15 per 5 kg/m²), and endometrioid (OR/RR=1.17 and 1.08 per 5 kg/m²) tumors. Overall, serous tumors were not associated with an increased risk in either study; however, the OCAC analysis included stratification by tumor grade and found an increased risk for low-grade serous tumors only (OR=1.13, per 5 kg/m²). An increased risk for OvCa has been observed between WHR and risk of mucinous tumors (HR per 0.05 unit increment = 1.19, 95% CI: 1.02-1.38), but not with serous, endometrioid or clear cell tumors.²⁵⁵ The large prospective NIH-AARP Diet and Health Study reported that obese women had an elevated risk of endometrioid OvCa

(RR=1.64, 95% CI: 1.00-2.70), but not for serous OvCa.¹⁷² Similarly, in the NHS, obesity was associated with increased endometrioid risk;³¹ however, in a systematic review, only the pooled analysis and one case-control study found BMI to be associated with an increased risk of endometrioid OvCa.²⁶⁰ Recent data from a pooled analysis of case-control and nested case-control studies further support the influence of obesity on OvCa risk among African Americans and White women; this risk appears to differ based on HRT use and histology²⁶¹.

In 2021, Baumeister et al.²⁶² evaluated the relationship between body fat and OvCa risk using updated data from the NIH-AARP Diet and Health Study which included 683 EOC cases (343 high-grade serous, 141 non-high grade serous) among 145,575 women aged 50-72 years, with a median follow-up of 12.6 years. No statistically significant associations were reported overall or by histology, and the authors concluded that “adult adiposity is not associated with ovarian cancer risk in post-menopausal women.” In 2022, a systematic review and meta-analysis by Ellwanger and colleagues²⁶³ was published on anthropometric factors and EOC risk. Data from 15 cohort studies and 26 case-control studies was included, and a total of 28,471 EOC cases were represented. The RR for the association between being overweight and obesity and overall EOC risk was 1.06 (95% CI: 1.00-1.12) and 1.19 (95% CI: 1.11-1.28), respectively. While overweight and obese pre-menopausal women demonstrated an elevated risk (RR 1.34, 95% CI: 1.03-1.75 and RR 1.51, 95% CI: 1.21-1.88, respectively), no association was observed with EOC risk for overweight and obese post-menopausal women (RR 1.00, 95% CI: 0.87-1.14 and RR 1.03, 95% CI: 0.82-1.31, respectively). This finding aligns with that from the NIH-AARP Study.²⁶² Subtype-specific analysis revealed an increased risk for mucinous (RR 1.44, 95% CI: 1.03-2.01) and clear cell (RR 1.82, 95% CI = 1.11-2.99) histologies, but not for serous (RR 1.12, 95% CI = 0.84-1.50;) and endometrioid disease (RR 1.24, 95% CI: 0.96-1.60). Finally, a recent umbrella review of meta-analyses also concluded that being overweight in adulthood increases the incidence of OvCa; histology-specific estimates were not provided.²⁶⁴

Since OvCa and other cancer types typically have a long latency period from initiation to manifestation, it is biologically plausible that early life weight or body size may influence OvCa risk many decades later during adulthood.²⁶⁵ Indeed, studies suggest that

higher birthweight is positively associated with OvCa risk overall (HR (95% CI): 1.51 (1.21-1.87) and serous OvCa (1.98 (95%CI: 1.47-2.67)).²⁶⁶ Additionally, girls with above average BMI at ages 7 and 13 years have been reported to have increased risks for OvCa overall and for clear cell, endometrioid, and mucinous cancers.²⁶⁷ In 2022, a meta-analysis of 10 studies (5 cohort and 5 case-control) published by Ding et al. explored the relationship between obesity in children and adolescents and EOC risk.²⁶⁸ Analyses demonstrated statistically significant associations between obesity in children and adolescents and OvCa risk overall (adjusted RR = 1.19, 95% CI: 1.11-1.28, $P < 0.001$). Significant associations were highest between obesity at age 17 and OvCa risk (RR = 1.49, 95% CI: 1.14- 1.94). Associations were consistently observed in cohort studies (RR = 1.29, 95% CI: 1.13 to 1.47, $P < 0.001$) and case–control studies (OR = 1.15, 95% CI: 1.06 to 1.25, $P = 0.001$) and for all geographic locations evaluated (e.g., America and Europe). Histology-specific estimates were not provided. In dose-response analyses, a trend was identified, with OvCa risk increasing as BMI increased, especially when BMI was $> 25.95 \text{ kg/m}^2$.

Importantly, biological plausibility exists for the involvement of excess adipose tissue in ovarian carcinogenesis by secreting adipokines, metabolic remodeling, and regulating the immune microenvironment.²⁶⁹ Since adiposity is a modifiable risk factor that may have long-term effects for OvCa, other cancers, and other chronic diseases, weight control and having a physically active lifestyle is important, especially earlier in life.

Gynecologic Surgery

Several gynecologic procedures appear to influence the risk for OvCa. It is well-established that among high-risk women, bilateral prophylactic oophorectomy or salpingo-oophorectomy (removal of the ovaries and the fallopian tubes) decreases OvCa risk by 80-90%.^{270,271} Numerous studies have identified a reduced risk of OvCa associated with a tubal ligation ranging from 30-40%,^{136,145,219,271-276} with the highest risk reductions observed among endometrioid and clear cell histotypes.^{32,275,277-280} Furthermore, the risk reduction from tubal ligation appears to last for at least 10-15 years, which argues against screening bias (due to selective removal of sub-clinical ovarian tumors).^{153,272,281,282} As such, fallopian tube removal (salpingectomy) may be recommended for OvCa prevention in women who are finished with childbearing and

choose to undergo elective pelvic surgery or hysterectomy.²⁸³ Indeed, in 2022, a systematic review by Kahn et al.²⁸⁴ concluded that bilateral salpingectomy was associated with an approximate 80% OvCa risk reduction and that it was safe, cost-effective, and not associated with an earlier age of menopause onset. Prospective studies were recommended to examine long-term survival outcomes in this patient population.

The association between hysterectomy (without oophorectomy) is not as clear. A systematic review and meta-analysis regarding the association between hysterectomy and risk of histologically-confirmed OvCa showed that there has been a temporal shift in this association, with a RR = 0.70 (95% CI 0.65-0.76) for a median year diagnosis before 2000, and a RR = 1.18 (95% CI 1.06-1.31) for post-2000).²⁸⁵ The authors suggested that this observation may be explained by the trend away from hysterectomy in younger women, along with the decline in oophorectomy rates and the use of estrogen-only hormone replacement therapy in women who have undergone hysterectomy.²⁸⁵ Similarly, a more recent pooled analysis of cohort studies³² also showed the absence of an inverse association between hysterectomy and OvCa risk overall, but did show a 40% risk reduction in clear cell cancers. Recently, a population-based longitudinal record linkage study in Western Australia aimed to explore the relationship between hysterectomy (without oophorectomy) and OvCa incidence.²⁸⁶ In an evaluation of more than 78,000 women who had the procedure, the authors found that hysterectomy was not associated with invasive OvCa risk overall (HR=0.98, 95% CI: 0.85-1.11) or with serous histology (HR=1.05, 95% CI:0.89-1.23).²⁸⁶ However, among the small subset of women with endometriosis or fibroids, hysterectomy was associated with a significantly reduced OvCa risk overall and across subtypes, a finding that warrants confirmation. In 2023, Ring et al.²⁸⁷ conducted a nationwide case-control study of the Danish Cancer Registry to investigate the association between hysterectomy and risk of EOC by histology and by history of endometriosis and menopausal hormone therapy (MHT) use. No association was detected between hysterectomy and EOC risk overall (OR=0.99, 95% CI 0.91-1.09), but a decreased risk of clear cell OvCa was observed (OR=0.46, 95% CI 0.28-0.78). Whereas stratified analyses showed that hysterectomy was associated with reduced risks for OvCa among women with endometriosis (OR=0.74, 95% CI 0.50-1.10) and among non-users of MHT (OR=0.87, 95% CI 0.76-1.01), long-term MHT users had an increased

OvCa risk (OR=1.20, 95% CI 1.03-1.39). Reasons for different results across studies may reflect different surgical approaches, indications or ages of women at surgery. Nonetheless, these results collectively suggest that for most women, having a hysterectomy without removal of ovaries does not substantially influence OvCa risk.

Although it is unknown how salpingectomy or tubal ligation reduces the risk of OvCa, it has been proposed that through retrograde menstruation (i.e., menstrual fluid flows backwards into the fallopian tubes instead of leaving the body through the vagina), endometrial tissue implants on peritoneal and ovarian surfaces (endometriosis) and becomes invasive, developing into endometrioid or clear cell ovarian carcinomas.^{15,288} This hypothesis is supported by epidemiological studies that show the strongest associations with tubal ligation and endometriosis for ENOC and CCOC. More recently, an alternate biologically rational mechanism has been proposed to explain the risk reduction afforded by tubal ligation. It has been suggested that tubal ligation induces quiescence in the epithelia of the fallopian tube fimbria.²⁸⁹ Using human samples and in vitro growth assays, Tiourin et al.²⁸⁹ showed that tubal ligation is associated with decreased presence and proliferation of progenitor cells in the distal fallopian tube epithelium. Given that HGSOC can initiate in distal fallopian tube epithelium, quiescence of epithelial progenitors in the fimbriated end of the tube may be a mechanism accounting for risk reduction of HGSOC following tubal ligation. In other words, tubal ligation decreases growth capacity of the fimbriated epithelium and supports risk reduction of HGSOC following tubal ligation. Taken together, the prospect of salpingectomy or tubal ligation, rather than prophylactic salpingo-oophorectomy, may serve as a more acceptable risk reduction procedure for pre-menopausal women, as it allows them to retain their ovaries.

Benign gynecologic conditions

Several gynecologic conditions have been evaluated as risk factors for OvCa, including polycystic ovarian syndrome (PCOS), endometriosis, and pelvic inflammatory disease (PID). PCOS is a multi-factorial disease often characterized by obesity, hirsutism, infertility and menstrual abnormalities. Due to unopposed endogenous estrogen and/or elevated androgens, women with PCOS have an increased risk for endometrial cancer. The association between PCOS and OvCa risk was investigated using data from the

Cancer and Steroid Hormone Study, a population-based case-control study.¹⁹² Among 476 histologically confirmed epithelial OvCa cases and 4,081 controls, 7 cases (1.5%) and 24 controls (0.06%) reported a history of PCOS (OR= 2.5 fold, 95% CI: 1.1-5.9).¹⁹² The limited data were insufficient for a consensus statement that PCOS is a risk factor for OvCa.²⁹⁰ In 2014, the first meta-analysis was conducted to examine gynecological cancers in women with PCOS younger than 54 years of age compared with controls of similar ages.²⁹¹ The risk of OvCa was not significantly increased (OR, 1.41, 95% CI, 0.93-2.15, $P < 0.11$). However, the authors pointed out that “available evidence is far from robust and variation in diagnostic criteria for PCOS, associated risk factors (particularly obesity), and selection bias in the studies likely influenced the findings.”²⁹¹ More recently, long and irregular menstrual cycles, a hallmark of PCOS, were not found to be associated with OvCa risk overall among 2,041 women with epithelial OvCa and 2,100 controls in the New England Case-Control Study (1992-2008) (OR = 0.97, 95% CI = 0.61-1.56).²⁹² However, menstrual cycle irregularity was associated with a decreased risk of high grade serous tumors and an increased risk of serous borderline tumors among women who had never used OCs and those who were overweight.²⁹² Further studies are clearly needed.

Endometriosis is one of the most common gynecological disorders, affecting 10-15% of women in reproductive years.²⁹³ Despite being considered a benign condition, endometriosis has been linked with OvCa in the medical literature since 1925. Sayasneh and colleagues²⁹³ conducted a systematic review of eight studies; seven reported an increased risk of OvCa, with effect sizes ranging from 1.3 to 1.9. The strongest associations between endometriosis and OvCa risk are evident among endometrioid and clear cell histologies,^{32,293,294} consistent with molecular data that supports endometrial epithelium as the origin of these subtypes.⁷ In addition, Pearce and colleagues²⁹⁵ identified an increased risk of low-grade serous OvCa (OR=2.11, 95% CI: 1.39-3.20) among women with endometriosis as well as for endometrioid (OR=2.04, 95% CI: 1.67-2.48) and clear cell cancers (OR=3.05, 95% CI: 2.43-3.84). The authors speculated that the processes of endometriosis and endosalpingiosis may result from a similar underlying host susceptibility to implantation of exfoliated Müllerian epithelial cells from the endometrium and fallopian tube. The association between endometriosis and

endometrioid and clear cell ovarian carcinomas may represent shared risk factors,²⁹³ genetic susceptibility²⁹⁶ and/or pathogenesis.²⁹⁷

In a pooled analysis of 9 case-control studies in the Ovarian Cancer Association Consortium (OCAC), Phung and colleagues²⁹⁸ recently evaluated the association between risk factors for OvCa and OvCa susceptibility and the association between talc and OvCa risk among women with vs. without self-reported endometriosis. Most risk factor-OvCa associations were similar when comparing women with and without endometriosis, and no statistically significant interactions were detected. Genital talcum powder use and use of long-term menopausal estrogen-only therapy was reported to increase OvCa risk, with a greater risk for those with endometriosis vs. those without (genital talcum powder: OR = 1.38, 95% CI, 1.04-1.84 vs. OR = 1.12, 95% CI, 1.01-1.25, respectively; ≥ 10 years of estrogen-only therapy: OR = 1.88, 95% CI, 1.09-3.24 vs. OR = 1.42, 95% CI, 1.14-1.76, respectively); interactions were not statistically significant. Findings should be interpreted with caution for several reasons. First, information on endometriosis history was based on self-report and was not medically conformed, potentially leading to misclassification of disease. Additionally, a significant proportion of the study population was missing data on genital talcum powder use. Data on genital talcum powder use was not collected in the Danish study and was missing for 41.4% of the US studies and for 5% of women in the Australian study. Only 79 OvCa cases with endometriosis reported use of genital talc, so the sample size was small. Moreover, the authors²⁹⁸ only adjusted for age, race/ethnicity, education level, and OCAC study. No well-established risk factors were included in models. For example, family history of OvCa in a first degree relative was reported to be a major risk factor among women without endometriosis in this cohort, yet it was not adjusted for in the risk prediction models.

PID causes inflammation of the endometrium, fallopian tubes and ovaries. Studies evaluating the association between PID and OvCa risk have yielded inconsistent results.^{299,148,300-302} In previous studies of PID and OvCa risk, some only considered invasive tumors,^{147,148,303} whereas others included both invasive and borderline tumors,³⁰¹ perhaps contributing to the inconsistent findings. In a nationwide population-based retrospective cohort study in Taiwan that included 32,698 patients with PID (verified by medical record) and an equal number of controls without PID, no increased OvCa risk

was observed (HR 1.33 (95% CI: 0.78-2.27)).³⁰² In the Danish MALOVA (MALignant OVarian tumor) case-control study of 2,300 women, PID history was associated with increased risk of ovarian borderline tumors, but not with invasive OvCa.³⁰⁴ Rasmussen et al.³⁰⁵ further evaluated borderline ovarian tumors in a cohort of more than 1.3 million Danish women and found that history of PID was associated with an 85% increased risk of serous borderline tumors, but not those of the mucinous subtype. In 2016, Rasmussen and colleagues³⁰⁵ published a pooled analysis of 13 case-control studies from the Ovarian Cancer Association Consortium that evaluated the association between self-reported PID and the risk of OvCa and borderline ovarian tumors. No association was observed between PID and OvCa risk overall (pooled OR=0.99, 95% CI: 0.83-1.19). However, a history of PID was associated with an increased risk of borderline ovarian tumors (pooled OR=1.32, 95% CI: 1.10-1.58); the risk increased in the presence of a history of at least 2 PID episodes (pooled OR=2.14, 95% CI: 1.08-4.24) and was nonsignificantly increased among women with low-grade serous borderline tumors (pooled OR=1.48, 95% CI: 0.92-2.38). Analyses were adjusted for several important confounders, such as age, parity, oral contraceptive use and family history of ovarian or breast cancer. However, most component studies of the pooled analysis were limited because they relied on self-reported history of PID. Misclassification of PID may occur when women inadvertently report bladder or vaginal infections as PID. Furthermore, as will be discussed shortly, most studies did not evaluate an infectious etiology to clinical PID.

A meta-analysis of 13 studies (7 cohort and 6 case-control) by Zhou et al.³⁰⁶ reported an association between PID and OvCa risk overall (RR 1.24, 95% CI: 1.06-1.44), particularly among Asian women (RR 1.69, 95% CI: 1.22-2.34). Results were not significant among Caucasian women (RR 1.18, 95% CI 1.00-1.39). The RR among cohort studies (which used hospital records to verify PID history) was statistically significant, but the RR for case-control studies (which used self-report) was not. PID history was associated with an increased risk for borderline ovarian tumors (RR 1.14, 95% CI: 1.25-1.63). No histology-specific estimates were provided. Of note, two case-control studies^{299,307} were included in both the pooled analysis³⁰⁵ and the meta-analysis.³⁰⁶

Taken together, if an association exists between PID and ovarian tumors, it is weak and is likely restricted to borderline ovarian tumors rather than invasive OvCa.

Importantly, given that *Chlamydia trachomatis* infection and *Neisseria gonorrhea* are the most important cause of PID in developed countries and remains underreported due to the often asymptomatic clinical presentation,³⁰⁸⁻³¹⁰ serologic testing for antibodies to *Chlamydia trachomatis* or *Neisseria gonorrhea* should have been performed to enhance the validity of the aforementioned studies and to evaluate these infectious diseases as etiologic factors associated with OvCa.

ii. Inconclusive or Insufficient Evidence to Support Causality

Sexually Transmitted Diseases (STDs)

STDs, which include bacterial infections such as *Chlamydia trachomatis* and *Mycoplasma genitalium*, and *Neisseria gonorrhea*, and viral infections including, *human papillomavirus (HPV)* and *herpes simplex virus 2 (HSV-2)*, have been associated with PID, salpingitis (tubal inflammation), and/or tubal infertility.³¹¹⁻³¹⁴ Studies have also shown the presence of viral and bacterial markers in human OvCa and fallopian tube tumor samples.³¹⁵ In 2003, Ness and colleagues³¹⁶ studied IgG antibodies to serovar D of *Chlamydia trachomatis* elementary bodies (EB) in 117 women with OvCa and in 171 age- and ethnicity-matched population-based control subjects and found that the probability of having OvCa was 90% higher in women with the highest levels, suggesting past or chronic persistent *Chlamydia trachomatis* infection may be a risk factor for OvCa. However, a subsequent study by Ness and colleagues³¹⁷ that was conducted in a larger sample of 521 OvCa cases and 766 controls using the same methodology did not support their earlier finding of elevated titers for antibodies to *Chlamydia trachomatis* among women with OvCa.

Given that STDs are associated with tubal pathologies and that the fallopian tube is the origin of a subset of serous OvCa, Fortner et al.³¹⁸ hypothesized that STDs may be risk factors for epithelial OvCa. To evaluate this hypothesis, the authors³¹⁸ measured antibodies indicating past infection with *Chlamydia trachomatis*, *Mycoplasma genitalium*, *HSV-2*, and antibodies against human papillomavirus oncogenes (L1 and E6+E7 oncoproteins of types 16, 18, and 45) using prediagnostic serum samples from 337 cases and 337 controls from the Nurses' Health Study (NHS) Cohort who were matched on year of birth (± 1 year) and menopausal status at diagnosis (pre-menopausal, post-menopausal or unknown). The study population for this nested case-control study had a

median age of 60 years old at baseline and most women (69%) were post-menopausal. The Pgp3-based antibody, the 'gold standard' serological marker for accurately measuring past or current *Chlamydia* infection status, was used in this study. Seropositivity to *Chlamydia trachomatis* infection was associated with a two-fold increased risk of OvCa (RR: 2.07, 95% CI: 1.15-3.43) using the laboratory cutpoint; risks were similar for women with invasive disease (RR:1.98, 95% CI: 1.21-3.23), invasive serous disease (RR: 2.31, 95% CI: 1.33-4.01) and borderline ovarian tumors (RR: 2.11, 95% CI: 1.04-4.28). Furthermore, risks persisted regardless of the antibody level. Statistically significant associations were *not* observed for antibodies to any other individual infections. When compared to women who were seronegative to all evaluated infections, seropositivity to *Chlamydia trachomatis* and any other infection (primarily *M. genitalium* or HSV-2) was associated with a 2.74-fold higher OvCa risk (95%CI:1.20-6.27), while *Chlamydia trachomatis* alone had a RR=1.88 (95% CI: 1.03-3.42). Upon excluding women who had undergone tubal ligation, the approximate two-fold increased risk of OvCa remained (RR: 1.94, 95% CI: 1.17-3.21). Associations did not differ by menopausal status at blood collection or diagnosis, age at blood collection, or ever use of oral contraceptives. Taken together, this study³¹⁸ suggests that past or current infection with *Chlamydia trachomatis* may be associated with OvCa risk among different subsets of women. However, a limitation of this study³¹⁸ is the lack of inclusion of data on variables such as sexual history and history of PID and/or other pelvic diseases and their treatment. Thus, results may be due to residual confounding by these covariates. However, strengths include a validated assay to assess antibodies indicating a history of infection using blood samples obtained approximately 7 years prior to diagnosis. Because antibodies are relatively stable in blood, misclassification of the exposure is expected to be minimal. Additionally, while it is known that an infection was present at or prior to blood collection, it is unclear when an infection initially occurred or whether subsequent infections manifested in the interval between blood collection and diagnosis, or what proportion of women with negative serology had a history of an infection but did not seroconvert. This would likely result in non-differential misclassification of the exposure because these issues would likely be similar among women who developed OvCa after blood collection and those who remained cancer free.

More recently, Trabert and colleagues³¹⁹ used the same laboratory methods as the study by Fortner et al.,³¹⁸ and reported a positive statistically significant association between antibodies to *Chlamydia trachomatis* (at different cutpoints) and OvCa risk in a population-based case-control study in Poland (244 cases; 556 controls) and in the prospective Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial (160 cases; 159 controls). In the Polish study, antibodies against Pgp3 were associated with elevated OvCa risk at the laboratory cutpoint (adjusted odds ratio [OR] 1.63, 95% CI 1.20–2.22) and at higher cutpoint thresholds ((cutpoint 2: 2.00, 1.38–2.89; cutpoint 3: 2.19, 1.29–3.73)). In the PLCO study, Pgp3 antibodies were significantly associated with an elevated OvCa risk only at cutpoint 2 (2.25, 1.07–4.71). In both the Poland and PLCO studies, antibodies against other measured infectious agents were not risk-associated. Similar to the NHS study,³¹⁸ the PLCO study collected blood samples prior to diagnosis of OvCa, reducing the potential that disease status affected immune functioning. OvCa risks did not differ substantially by serous/non-serous histology for either the Poland or PLCO. Study limitations included not testing for antibodies to *Neisseria gonorrhoeae*, a known cause of PID and lack of adjustment for treatment of *Chlamydia* infections with antibiotic use. Additionally, the authors could not rule out that the waning of marker positivity observed in the PLCO study (older mean age at blood draw) may have been due to reduced persistence of the antibody response over a long period; however, results did not change dramatically in analyses excluding women age 70 years or older.

Collectively, findings from the recent studies by Fortner³¹⁸ and Trabert³¹⁹ support the hypothesis that *Chlamydia trachomatis* infection may be an etiologically relevant phenomenon for OvCa. If confirmed, these findings suggest that *Chlamydia* prevention and early detection efforts (which may include vaccine development) could present an opportunity to reduce OvCa risk. Importantly, there is biological plausibility for a possible *Chlamydia trachomatis*-OvCa association based on several lines of molecular data. For example, *Chlamydia trachomatis* has been shown to induce double-stranded DNA breaks, interfere with the DNA damage response, and inhibit apoptosis in the host cell.^{320,321} Moreover, this infection may indirectly influence OvCa development by causing inflammation in the genital tract in the form of salpingitis and other tubal pathologies, including tubal damage-inducing PID.³¹¹ The pathogenesis of such irreversible and

permanent tubal damage is a consequence of innate and adaptive immune responses to ongoing or repeated infections.³²² In particular, the heat shock protein of *Chlamydia* (cHSP60) is speculated to induce pathogenic immune responses that cause a local proinflammatory response in fallopian tube epithelia, resulting in scar formation and tubal occlusion.^{311,322} This may be particularly relevant for the serous histotype, as the fallopian tube has been identified as the origin of the majority of these tumors, with serous tubal intraepithelial carcinomas (STICs) as a suggested precursor that can rapidly spread to the ovarian surface and peritoneum.³²³ Furthermore, upper genital tract chlamydial infections have been reported to cause adhesions between the fallopian tube and ovary, leading to transfer of tubal-initiated cells to the growth-promoting microenvironment in the ovary^{459,465}. To better understand key steps in the development of *Chlamydia*-induced tubal inflammation (salpingitis), Kessler and colleagues³²⁴ established long-term organoid cultures from human primary fallopian tube cultures with genital serovars D,K and E, which are the main drivers of tubal pathology in vivo. Sustained age-related changes in DNA methylation and sustained upregulation of EZH2 (a marker of higher stemness potential) were observed in long-term culture, consistent with reports of EZH2 overexpression in HGSOC tissue and a role for *chlamydia*-induced transition to invasion in host cells.³²⁵ Study results suggest a potential role of chronic *Chlamydia* infection as an epigenetic modulator and a possible driver of cell transformation and HGSOC initiation.³²⁴

Interestingly, even though *Chlamydia trachomatis* infections promote disturbances in the fallopian tube,³²⁶ early studies did not regard these infections as risk factors for primary fallopian tube carcinomas (PFTC).³²⁷ Serological case-control studies of prior infections of *Chlamydia trachomatis* or human papillomavirus (HPV) on the risk of PFTC^{328,329} did not confirm earlier thoughts of a prior salpingitis as a promoter for PFTC. Instead, various hormonal, reproductive and genetic factors have been postulated to play a role in increasing PFTC risk^{330,331,326}. However, because *Chlamydia trachomatis* infection is common at a young age and PFTC develops decades later, the possibility remains that *Chlamydia trachomatis* contributes to PFTC development. More recently, a retrospective study in Egypt that performed semiquantitative qRT-PCR on 67 ovarian and fallopian tube cancers showed that *Chlamydia trachomatis* DNA was present in 84%

(n=21) of high-grade tubal serous cancers, 16.7% of serous ovarian cancers, and 13.3% of benign control tissues.³³² Quantitative measurement of DNA load revealed that levels of *Chlamydia trachomatis* were significantly higher in the tubal serous cancers compared with the ovarian serous cancers and controls ($P<0.0005$). This study³³² was the first to report on the detection of *Chlamydia trachomatis* DNA in the pathogenesis of PFTC and OvCa. Findings³³² differ from prior studies that relied on serology in ovarian and tubal cancers,^{200,201} but are in line with studies that revealed the presence of *Chlamydia trachomatis* DNA in invasive ovarian tumors.³³³ Taken together, future studies are warranted to investigate molecular mechanisms that may link *Chlamydia trachomatis* DNA to the initiation and progression of PFTC and ovarian tumors. Future research may also entail study of coinfection of *Chlamydia trachomatis* with HHV-6 (a betaherpesvirus); a synergistic relationship between the pathogens has been suggested to induce transformation to cancer in ovarian cells.³³⁴

Cigarette smoking

Cigarette smoking was initially not believed to be a risk factor for OvCa,^{166,335-337} but results from more recent studies suggest this is most likely because analyses were not conducted separately for histologic subtypes. Indeed, smoking appears to increase the risk for mucinous OvCa in a dose-response manner, but not other subtypes.^{24,28,32,338} In 2012, a meta-analysis of 51 epidemiological studies concluded that current smokers have a 50% increase in invasive mucinous OvCa risk and an over two-fold increase in borderline mucinous OvCa risk (summary RR=2.25, 95% CI: 1.64-3.08) compared to never smokers, but no increased risk of serous (0.96, 95% CI: 0.87-1.06) or clear cell (0.80, 95% CI: 0.63-1.01) cancers and lower risk of endometrioid cancers (0.82, 95% CI: 0.71-0.95).³³⁹ In another meta-analysis, the risk of mucinous cancer increased in a dose-response relationship with amount smoked, but returned to that of never smokers within 20-30 years of stopping smoking.³⁴⁰ Histologically, mucinous ovarian tumors resemble mucinous gastrointestinal cancers, some of which (pancreatic gastric and colorectal cancers) have also been associated with smoking.^{340,341} Collectively, these findings suggest that risk of OvCa is another reason to avoid cigarette smoking.

Exercise and Physical Activity

The general health benefits of exercise are well established and it has been hypothesized that a specific effect on OvCa may occur through mechanisms such as reduction of adipose tissue (and therefore estrogen and androgen levels) or reduced ovulation.^{342,343} To date, at least 30 epidemiologic studies have investigated physical activity and OvCa risk, including prospective cohort studies,³⁴⁴⁻³⁵⁷ historical cohort studies,^{358,359} population-based case-control studies³⁶⁰⁻³⁶⁹ and hospital-based case-control studies.³⁷⁰⁻³⁷² Results are not entirely consistent, but a 2007 meta-analysis estimated a nearly 20% lower risk for the most active women compared to the least active (pooled relative risk = 0.81, 95% CI: 0.72-0.92).³⁶⁵ Most studies that measured physical activity across the lifespan reported consistent null findings^{351,354,362,365,373} or risk reductions^{361,363,364,370} in each age period. Similarly, prolonged sedentary behavior,³⁷³ high levels of total sitting duration^{355,357,374,375} and chronic recreational physical inactivity^{356,368} have all been noted to increase risk. The benefit of physical activity does not appear to vary by histological type^{357,368} but there are insufficient data to draw firm conclusions.^{364,367} Considering the benefits of exercise on weight control, bone density and heart disease, the promotion of regular activity should be encouraged.

Diet and Nutrition

Despite numerous analytical epidemiologic studies, whether diet affects the risk of OvCa is largely unresolved. Factors that have been evaluated include intakes of vegetables, whole grain foods and low-fat milk, with higher intakes generally associated with lower risk.³⁷⁶ Associations with specific fats and oils, fish and meats and certain milk products are inconsistent, and no firm conclusions can be made. For example, although one meta-analysis of two large cohort studies found that women with a high intake of saturated fats had elevated OvCa risks (HR=1.21 (95% CI: 1.04-1.41))³⁷⁷ and another meta-analysis³⁷⁸ suggested that dietary total, trans, saturated and partially monounsaturated fat and cholesterol intake are positively associated with an increased risk of OvCa, two large prospective studies of Nurses' Health Study (NHS) and NHSII participants did not observe clear associations between dietary fat and OvCa risk.³⁷⁹ Studies on meat consumption are not consistent.³⁸⁰⁻³⁸² A large prospective study found that women in the highest intake quartile of dietary nitrate had an increased risk of OvCa

(HR= 1.31, 95% CI: 1.01-1.68, and p-value for trend = 0.02). Similarly, the association between coffee and tea intake and OvCa has been inconclusive,^{147,149,337,383-388} with a recent systematic review and meta-analysis of 20 case-control studies³⁸⁹ showing no significant association between total caffeine intake and OvCa risk (OR=0.89, 95% CI 0.55 to 1.45).

Another area with conflicting findings relates to vitamin D. Although the majority of vitamin D is produced in the skin from UV-B exposure,³⁹⁰ it is also partly obtained from our diet or dietary supplements. Experimental studies have shown that a metabolized form of vitamin D inhibits cell proliferation in OvCa cell lines and induces apoptosis.³⁹¹ However, epidemiologic evidence that vitamin D status influences OvCa risk is inconsistent. One systemic review concluded that there is no strong evidence that Vitamin D decreases OvCa risk,³⁹² and a meta-analysis of ten longitudinal studies³⁹³ and other cohort studies³⁹⁴ reached a similar conclusion. In the meta-analysis, the protective effect was evident in seven of the ten studies and the pooled estimate was a 17% reduced risk with increasing 25(OH)D levels; however, the pooled estimate was not statistically significant (RR= 0.83, 95% CI: 0.63–1.08).³⁹³ Using data from the New England case-control study (1,909 cases and 1,989 controls), Merritt et al.³⁹⁵ examined dairy foods and nutrients in relation to risk of OvCa and its histological subtypes and observed a reduced overall risk of OvCa with high intake of total calcium [Quartile 4 (Q4, >1,319 mg/day) vs Quartile 1 (Q1, <655 mg/day), OR = 0.62, 95% CI = 0.49-0.79], especially for serous borderline and mucinous tumors. High intake of total vitamin D was not associated with OvCa risk overall, but was inversely associated with risk of serous borderline (Q4, >559 IU/day vs Q1, <164 IU/day, OR = 0.51, 95% CI = 0.34-0.76) and endometrioid tumors (Q4 vs Q1, OR = 0.55, 95% CI = 0.39-0.80).

Several studies have also investigated whether single nucleotide polymorphisms (SNP) in the vitamin D receptor (VDR) gene and/or other vitamin D biosynthesis pathways are associated with circulating 25-hydroxyvitamin D [25(OH)D] concentrations and/or OvCa risk.^{396-398,399} For example, Prescott et al.³⁹⁸ evaluated VDR genetic associations by high vs low predicted 25(OH)D in the Nurses' Health Studies (562 cases, 1,553 controls) and New England Case-Control study (1,821 cases, 1,870 controls) and found that rs7975232 genotype increased OvCa risk (OR = 1.38) among women with high

predicted 25(OH)D, but not among women with low levels ($P \leq 0.009$). In contrast, Ong et al.³⁹⁹ included 31,719 women of European ancestry (10,065 cases (4,121 HGSOE) and 21,654 controls) from the Ovarian Cancer Association Consortium, and observed that *lower* 25(OH)D concentrations were associated with increased risk for OvCa overall (OR (95% CI): 1.27 (1.06 -1.51)) and for HGSOE (1.54 (1.19-2.01)). These findings³⁹⁹ suggest that increasing plasma vitamin D levels may reduce OvCa risk. Despite these studies, the relationship between vitamin D, genetic variants and OvCa risk continues to remain uncertain due to conflicting findings.

Aspirin and Non-Steroidal Anti-Inflammatory Drugs

Animal and *in vitro* studies suggest that aspirin may inhibit the growth of OvCa, but the data are mixed.⁴⁰⁰⁻⁴⁰² Several prospective^{403,404} and case-control⁴⁰⁵⁻⁴⁰⁹ studies have observed an inverse association between aspirin and non-steroidal anti-inflammatory drugs (NSAIDs) and OvCa incidence, although other studies have reported no association.⁴¹⁰⁻⁴¹⁶ Prizment and colleagues⁴⁰⁴ investigated these drugs using data from a prospective cohort of approximately 20,000 women from the Iowa Women's Health Study. Compared to women who reported no use of aspirin, the RR of OvCa for those who used aspirin <2, 2-5 times, and ≥ 6 times per week were 0.83, 0.77, and 0.61, respectively (P trend=0.04), but no association was observed between NSAID use and OvCa risk. In the Nurses' Health Study I and II,⁴⁰³ regular use of NSAIDs (hazard ratio 0.81, 95% CI: 0.64-1.01) and aspirin use were not statistically significant (hazard ratio 1.11, 95% CI: 0.92-1.33). No dose-response relationship with increased frequency or duration of use was observed, and results did not differ when stratifying by tumor histology.⁴⁰³ A population-based case-control study conducted by Lo-Ciganic (2012)⁴¹⁷ in western Pennsylvania, eastern Ohio and western New York state included 902 women with incident epithelial OvCa who were diagnosed between February 2003 and November 2008, along with 1802 matched controls. Regular use (at least two tablets per week for six months or more) of aspirin, non-aspirin (NA)-NSAIDs, and acetaminophen before the reference date (nine months before interview date) was assessed by in-person interview. Decreased risks were observed among continuous aspirin users (OR=0.71 (0.54, 0.94)) and among those with a low-standardized daily dose (OR=0.72 (95% CI: 0.53, 0.97)). No associations were detected among women using NA-NSAIDs or acetaminophen.⁴¹⁷ It is worth noting that

acetaminophen and NA-NSAIDS are often used interchangeably, but acetaminophen has weak anti-inflammatory properties and may have gonadotropic effects, possibly supporting different observed associations and mechanisms of action.

In 2013, Baandrup and colleagues⁴¹⁸ published a systematic review and meta-analysis of observational studies (14 case-control (11 population-based, 3 hospital-based and 7 cohort)) that had evaluated aspirin and non-aspirin (NA) NSAIDs-OvCa risk associations before 2012. Exposure assessment in the component studies was primarily ascertained via interviews or self-administered questionnaires. The definition of “regular use” varied greatly among studies and ranged from “any use” to “four days or more per week for at least 6 months.” All component studies matched on age or adjusted for it, and most adjusted for parity, oral contraceptive use and family history of ovarian or breast cancer. In a combined analysis of invasive and borderline ovarian tumors, protective associations were detected between aspirin (RR=0.93, 95% CI: 0.84-1.02) and NA-NSAIDS (RR=0.94 (95% CI: 0.84-1.06)) and OvCa risk, but they were not statistically significant. Marked heterogeneity was observed between studies ($I^2=50.6\%$, $p<0.01$), and persisted when restricting to case-control studies and case-control study type (population- or hospital-based). When restricted to invasive cases only, risk was reduced and was statistically significant among aspirin users (RR= 0.88, 95% CI:0.79-0.98), and heterogeneity was reduced ($I^2=16.2\%$, $p=0.29$). Although risk was also reduced among NA-NSAID users, results were not statistically significant (RR=0.94, 95% CI:0.84-1.06) and between-study heterogeneity was high ($I^2=63.4\%$, $p<0.01$). When focusing on the maximum dose of aspirin and NA-NSAIDs, no dose response was observed. In summary, this meta-analysis did not find statistically significant associations between aspirin and non-aspirin NSAID use and OvCa risk. On the other hand, a pooled analysis published in 2014 of 12 case-control studies in the Ovarian Cancer Association Consortium⁴⁰⁵ found aspirin use was associated with a reduced risk of OvCa (OR=0.91, 95% CI: 0.84-0.99), especially among daily users of low-dose (<100 mg) aspirin (OR=0.66, 95% CI=0.53-0.83). Recently, Barnard et al.⁴¹⁹ showed a reduced risk of OvCa among regular users of low-dose aspirin but also found an *increased* risk among long-term users of NA NSAIDS. Finally, in a recent prospective analysis of 13 case-control studies in the Ovarian Cancer Cohort Consortium,⁴²⁰ women who used aspirin almost daily (≥ 6 days/wk) vs

infrequent/non-use had a 10% reduction in OvCa risk (rate ratio [RR] = 0.90, 95% confidence interval [CI] = 0.82 to 1.00, $P = .05$). This was among women who had used aspirin for less than 10 years. However, since the RR includes the null value of 1, there is insufficient evidence to conclude that the groups are statistically significantly different. Frequent use (≥ 4 days/wk.) of aspirin (RR = 0.95, 95% CI = 0.88 to 1.03), NSAIDs (RR = 1.00, 95% CI = 0.90 to 1.11), or acetaminophen (RR = 1.05, 95% CI = 0.88 to 1.24) was not associated with risk. Risk estimates for frequent, long-term (10+ years) use of aspirin (RR = 1.15, 95% CI = 0.98 to 1.34) or NA NSAIDs (RR = 1.19, 95% CI = 0.84 to 1.68) were elevated but statistically insignificant. Longer duration of use was *not* associated with further risk reduction. Histotype-specific analyses did not reveal statistically significant associations for any particular histology. The authors reported that the “observed potential elevated risks for 10+ years of frequent aspirin and NSAID use require further study but could be due to confounding by medical indications for use or variation in drug dosing.” It is also worth mentioning that this finding among long-term users is based on very small numbers of exposed cases. Taken together, that evidence suggests that use of NSAIDS is not inversely associated with the incidence of OvCa as may be expected if the etiology was related to chronic inflammation. Of note, the authors⁴⁰⁵ did not report on the prevalence of other factors linked to chronic inflammation (such as PID, *Chlamydia* and endometriosis) in this study.

In an Australia-wide case-control study of 1,576 women with invasive and borderline ovarian tumors and 1,509 population-based controls published in 2008, Merritt et al.³⁰⁷ evaluated the potential role of chronic local ovarian inflammation in the development of the major histologic subtypes of OvCa. In their study, talc use was associated with a weak increased OvCa risk for serous ($OR_{adjusted} = 1.21$, 95% CI: 1.03-1.44) and endometrioid subtypes ($OR_{adjusted} = 1.18$, 95% CI: 0.81-1.70), though the latter finding was not statistically significant. No clear dose response with increased use was identified. Other risk factors potentially associated with ovarian inflammation (pelvic inflammatory disease, human papilloma virus infection) were only associated with endometrioid and clear cell OvCa among women with a history of endometriosis. Regular use of aspirin and NSAIDS was inversely associated with the risk of borderline mucinous

tumors only. The authors concluded that chronic inflammation does not appear to play a major role in the development of OvCa.

Using data from the NIH-AARP Diet and Health Study (1995-2011) and the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial (1993-2009), Hurwitz and colleagues⁴²¹ evaluated whether age, BMI, smoking status, physical inactivity, and family history of cancer modify the effect of daily aspirin use (≥ 5 days/week) on the risk of colorectal, ovarian, breast, endometrial, and aggressive prostate cancer. Daily aspirin users experienced a 15% decreased risk for colorectal cancer (HR: 0.85, 95% CI 0.80–0.89). For OvCa, no significant association was detected overall (HR: 0.93, 95% CI 0.80–1.08), but risk reductions strengthened among obese women (HR: 0.72, 95% CI 0.52–0.98, p -interaction = 0.12). For breast, endometrial, and aggressive prostate cancer, associations were null or weak, and significant effect modification was not detected. Hurwitz et al.⁴²² also specifically evaluated the association between daily, low-dose aspirin and OVCa risk using data from PLCO, and observed that aspirin use was not significantly associated with OvCa risk (HR: 0.93, 95% CI: 0.72-1.21). Finally, Hurwitz and colleagues from the Ovarian Cancer Association Consortium (OCAC)⁴²³ evaluated whether the association between frequent aspirin use and OvCa risk may be modified by genetic susceptibility to OvCa using a polygenic score (PGS). Their⁴²³ pooled analysis of 8 case-control studies from OCAC included 4,476 cases with non-mucinous OvCa and 6,659 controls and revealed a 13% reduced risk of non-mucinous OvCa (OR: 0.87 [95%CI, 0.76-0.99]). Associations did not differ by PGS categories (all P interactions $>.05$). Collectively, I agree with the authors' assessment that frequent aspirin use may lower OvCa risk regardless of an individual's genetic susceptibility to OvCa. Taken together, data remain inconclusive regarding the association between aspirin use and OVCa risk. Future work should continue to explore the possible role of aspirin use for OvCa prevention among individuals who are at higher risk for the disease by building upon mechanistic studies in this area.^{424,425}

Metformin

A growing body of evidence supports a role for the anti-diabetic agent metformin in the prevention and treatment of multiple cancers.⁴²⁶ A case-control study including 1,611 incident OvCa cases was performed using the UK-based General Practice

Research Database.⁴²⁷ Long-term use (≥ 30 prescriptions) of metformin (and not sulfonylureas or insulin) was associated with a trend towards reduced OvCa risk (OR=0.61, 95% CI: 0.30-1.25), but the results were not statistically significant.⁴²⁷ Additional studies have observed decreased OvCa incidence and mortality among metformin-treated groups,⁴²⁸ but validation studies are needed. In light of anti-cancer effects of metformin that have been observed using OvCa cells,⁴²⁹ the potential for use of metformin as a chemopreventive agent merits further exploration.

Diabetes mellitus

Whereas an early meta-analysis of 7 case-control studies and 11 cohort studies reported a statistically significant 17% increase in the risk of OvCa among women with diabetes (of unknown type),⁴³⁰ there was moderate between-study heterogeneity, and results were not replicated in a newer population-based cohort study.⁴³¹ In a more recent meta-analysis of 13 studies that evaluated type II diabetes/diabetes mellitus, there was an increased risk of OvCa RR=1.24 (95%CI: 1.06-1.44) but heterogeneity was high ($P < 0.001$, $I^2 = 81.8\%$). It is possible that the heterogeneity may be attributed to differences in diabetes treatments since it has been suggested that metformin may have a protective role while insulin and sulfonylureas may confer increased risk.⁴²⁷

Alcohol Use

Alcohol consumption increases circulating concentrations of androgens, estrogens and other sex hormones in serum and urine and has been linked to increased risk of breast cancer.^{432,433} Studies of alcohol use and OvCa are inconsistent, with null associations,^{143,166,337,385,434-437} evidence for increased risk^{149,438,439} and decreased risk.⁴⁴⁰⁻⁴⁴² There have been efforts to resolve the observed inconsistency by quantifying risk by the type of alcohol consumed (wine, beer or spirits),^{439,440,443} histologic subtype of the tumor,^{439,440,442} or by other potential modifiers, such as dietary fiber intake.⁴⁴⁴ In a large population-based case-control study,⁴⁴⁵ consumption of beer (not liquor or wine) during early adulthood (20-30 years of age) was associated with a moderately increased risk of invasive OvCa, with the association limited to serous tumors (OR 1.52, 95% CI: 1.01-2.30), although results for other histological subtypes were based on sparse data. This risk was associated with regular consumption (1 or more drinks per day), and there was no evidence of a dose-response relationship. Data from the Netherlands Cohort

Study on Diet and Cancer found no association between alcohol consumption in the form of wine, beer or spirits and OvCa risk.⁴⁴⁶ A pooled analysis of 10 cohort studies that included more than 500,000 women and 2001 incident OvCa cases also observed no association between total alcohol intake (pooled multivariate RR=1.12, 95% CI: 0.86-1.44 comparing ≥ 30 to 0 g day of alcohol) or alcohol intake from wine, beer or spirits and OvCa.⁴⁴⁷ There was no association (OR=1.13, 95% CI: 0.92-1.38) between wine consumption and OvCa risk in a meta-analysis of 10 studies (3 cohort and 7 case-control studies) with 135,871 women, including 65,578 wine drinkers.⁴⁴⁸ Based on these data, it seems that if alcohol intake does influence the risk of OvCa, the magnitude is small and possibly limited to particular histologic subtypes.

Asbestos

Asbestos is the generic commercial designation for naturally occurring mineral silicate fibers of the serpentine and amphibole families. Serpentine silicates are classified as “sheet” silicates and include chrysotile (known as “white asbestos”) while amphibole silicates include amosite (also known as “brown asbestos”), crocidolite (also known as “blue asbestos”), anthophyllite, tremolite and actinolite.⁴⁴⁹ Crocidolite has been reported to pose a higher risk of lung cancer and to cause more deaths in comparison to the more common chrysotile.⁴⁴⁹ Epidemiologic data have consistently demonstrated an association between all forms of asbestos and lung cancer and mesothelioma, and asbestos has been classified as “carcinogenic to humans” (group 1) by IARC.⁴⁵⁰ On the other hand, published literature examining the association between asbestos and OvCa is limited as will be outlined below and should be interpreted with caution despite the IARC working group’s claim that a causal association between exposure to asbestos and OvCa is clearly established.⁴⁴⁹

Most of what is known regarding a possible link between asbestos and OvCa stems from occupational cohort or environmental studies. Most of these studies report the standardized mortality ratio (SMR), which is a ratio between the observed number of deaths in a study population and the number of deaths that would be expected, based on the age- and sex-specific rates in a standard population and the population size of the study population by the same age/sex groups. If the ratio of observed-to-expected deaths

is greater than 1.0, there is said to be “excess deaths” in the study population. If the SMR is equal to 1.0, the number of observed deaths equals that of expected cases.

The primary exposure to asbestos in those studies occurred by inhalation in occupations such as manufacturing, mining, milling, construction and asbestos insulation, in which the workforce has primarily been male.^{451,452} The sample size of the women included in these studies is very small, and the outcome is subject to disease misclassification since peritoneal mesotheliomas can be incorrectly classified as OvCa. Furthermore, literature based on occupational exposure cannot be readily extrapolated to studies of perineal exposure; it is like comparing apples and oranges. And the studies only crudely estimated the intensity of asbestos exposure, and most did not adjust for possible confounders such as reproductive and lifestyle factors. Below, I discuss a sampling of studies assessing a link between asbestos and OvCa.

Newhouse et al.⁴⁵³ identified nine deaths associated with ovarian neoplasms (3.6 expected) from a cohort of 922 women first employed at an asbestos factory between 1933 and 1942 and followed for more than 38 years. Of these nine cases, five occurred among women who were exposed for more than two years (0.9 cases were expected). A later study by Berry and colleagues,⁴⁵⁴ which evaluated asbestos workers in east London, found excesses of cancer of the ovary (RR= 2.53), but there was no consistent dose response among the nine total deaths (of less than four expected). It is noteworthy that there were no ICD codes for mesothelioma at the time of the death of these patients. Thus, possible sources of bias included the retrospective identification of patients by ICD code and lack of robustness of the pathologic review.

In 1982, Acheson et al.⁴⁵⁵ published on their evaluation of two groups of women exposed to asbestos while manufacturing gas masks in Lancashire before and during World War II. One group (in Blackburn) was involved primarily in the manufacture of civilian respirators (containing chrysotile), and the other (in Leyland) mainly created respirators for the armed forces (containing crocidolite). Excess mortality attributed to lung cancer and OvCa were found only at the factory in Leyland exposed to crocidolite, but the issue of disease misclassification was identified as a possible explanation for the excess risk reported for OvCa, since the malignancies at issue may have been misclassified as peritoneal mesotheliomas. Reid and colleagues⁴⁵⁶ evaluated 2,552

women and girls who were exposed to crocidolite asbestos domestically or from their environment (via dust, as opposed to in the workforce) when living in the blue asbestos mining and milling township of Wittenoom, Australia between 1943 and 1992. Quantitative measurements of asbestos exposure were derived from periodic dust surveys undertaken in the industry and around the township. Death records were obtained for the period 1950-2004. SMRs were calculated to compare the Wittenoom women's mortality with that of the Western Australian female population. Of a total of 425 deaths that occurred, 9 cases of OvCa were reported, which represented a non-statistically significant increase (SMR = 1.52, 95% CI: 0.69, 2.88). The study is limited in that exposure measurements were assigned to each woman in the cohort based on environmental monitoring during their period/timeframe of residence at Wittenoom. Thus, a woman's actual exposure may have been very different from the assigned exposure. Additionally, information that would help to gain a more accurate picture of the women's asbestos exposure (such as whether the women lived with or washed the clothes of an asbestos worker) was not always available. A later study by these same co-authors,⁴⁵⁷ which examined the incidence of gynecologic cancers after exposure to blue asbestos, revealed a standardized incidence ratio (SIR) of 1.27 that was not statistically significant among the Wittenoom women compared with the Western Australian population. Wignall et al.⁴⁵⁸ also reviewed observed versus expected rates of cancers in 500 women who assembled gas masks in World War II and found the number of women with OvCa to be higher in the group of workers exposed versus expected controls. The authors⁴⁵⁸ acknowledged the risk of misdiagnosis by pathology, but only attempted histologic review of three out of 500 cases, with one of the three samples found to be primary peritoneal malignant mesothelioma. This finding exemplifies the high potential for misclassification and the importance of additional histology review.

In Germany, Rosler and colleagues⁴⁵⁹ conducted a cohort study involving 616 female workers with a history of asbestos exposure. Mortality from lung cancer and mesothelioma was increased significantly over the expected value, but the same was not true for OvCa, with 2 observed and 1.8 expected cases and a SMR = 1.09 (95% CI: 0.13-3.95). Vasam-veuvonen and colleagues⁴⁶⁰ linked job titles of occupationally active Finnish women (n = 892,591) at the time of the 1970 census with incidence of OvCa using

Finnish Cancer Registry data from 1971-1995. Elevated risks for aromatic hydrocarbon solvents (SIR of 1.3 (95% CI 1.0, 1.7)), leather dust (1.4; 0.7, 2.7), man-made fibers (1.3; 0.9, 1.8), and high levels of asbestos (1.3; 0.9, 1.8), and diesel (1.7; 0.7, 4.1), and gasoline (1.5; 1.0, 2.0) were identified, but *none* of these exposures was significantly associated with OvCa risk.

In Italy, Germani⁴⁶¹ evaluated cause-specific mortality of all Italian women compensated for asbestosis. In a total cohort of 631 subjects, 277 deaths occurred. Cause-specific SMRs were significantly increased for the ovary (SMR of 477), although only 9 observations of OvCa were observed. The type of fiber the women were exposed to was not reported. Ferrante et al.⁴⁶² reported on a cohort of 1,780 wives of asbestos cement factory workers in Italy (exposed to a mix of crocidolite and chrysotile) but did not show a statistically significant increase in mortality for OvCa (11 observed vs 7.7 expected deaths). In another study of Italians by Mangani that was published by the same group⁴⁶³ that focused on asbestos cement workers *after* cessation of asbestos exposure, ovarian malignancies were more frequent than expected ($p < 0.01$) with 9 observed cases and 4 expected (SMR: 227.3, 95% CI: 103.9, 431.5). Most recently, Pira et al.⁴⁶⁴ reported on an Italian cohort of 894 male and 1,083 female textile workers with heavy asbestos exposure (primarily chrysotile, but crocidolite was present) who were employed between 1946 and 1984. The authors followed the workers until 2004 and reported SMRs of 29.1 (95% CI: 21.5-38.6) for peritoneal cancer, 2.96 (95% CI: 2.50-3.49) for lung cancer, 33.7 (95% CI: 25.7-43.4) for pleural cancer, and 3.03 (95% CI: 1.69-4.99) for OvCa.⁴⁶⁴ Higher SMRs were reported in women compared to men for pleural and peritoneal mesothelioma, and the authors hypothesized that the women in the reference population had a lower rate of these neoplasms due to the lower prevalence of prior asbestos exposure. In contrast to lung cancer, where the SMR declined after 35 years since last exposure, there was no evidence of a decrease for peritoneal and pleural cancer in relation to time since last exposure, possibly due to latency of subsequent mesothelioma risk.⁴⁶⁴ In a pooled analysis of 13,076 workers (including 18.1% women) from 21 Italian asbestos-cement factories published by Luberto et al. in 2019,⁴⁶⁵ significant excesses in mortality were observed for malignant neoplasms of the peritoneum (SMR: men 14.19; women 15.14), pleura (SMR: 22.35 and 48.10), lung (SMR: 1.67 and 1.67), and ovary (in the highest

tertile of exposure SMR 2.45). Limitations of this study include the fact that asbestos exposure was not assessed on an individual basis and failure to include medical records to confirm diagnoses.⁴⁶⁵

In the pulp and paper industry, large quantities of asbestos have been used as an insulation material in boilers and in certain rolling machines. As such, Langseth and Kjaerheim⁴⁶⁶ evaluated the association between OvCa risk and exposure to asbestos (and talc and total dust) in a nested case-control study of Norwegian pulp and paper workers comprising 46 OvCa cases and 179 controls. No statistically significant associations were found for asbestos (OR= 2.02, 95% CI: 0.72-5.66), talc (1.10 (95% CI: 0.56, 2.18) or dust 0.77 (95% CI: 0.35,1.58)) among workers. Household exposure to asbestos through others living in the same home or work clothes brought home by men were not risk-associated, with OR=0.82 and 0.86, respectively. Additionally, hygienic talc use (on diapers, sanitary napkins, or husband's use in genital area) was also not significantly associated with OvCa risk (OR=1.15, 95% CI: 0.41, 3.21).

Findings from meta-analyses

Two systematic reviews and meta-analyses with different conclusions have been conducted over the past decade to evaluate the asbestos-OvCa association.^{451,452} A study by Camargo et al.⁴⁵¹ investigated occupational cohorts with "well-documented" exposure of asbestos, while excluding case-control studies based on jobs and industries reported to have limited documentation of asbestos exposure.^{148,466,467} The authors⁴⁵¹ also excluded studies conducted among workers predominantly exposed to known or suspected carcinogens other than asbestos, such as the study by Vasama-Neuvonen⁴⁶⁰ (which is cited as supporting an asbestos-OvCa association in the IARC monograph⁴⁶⁸). Only one included study⁴⁵⁷ reported on OvCa incidence, and the remaining 17 were based on mortality, for a total of 125 OvCa deaths and one incident cancer case in the main analysis. The overall pooled SMR estimate for OvCa was 1.77 (95 % CI: 1.37, 2.28), with moderate heterogeneity among studies (P=0.06). Effect estimates were stronger for Italian and Polish cohorts compensated for asbestosis, cohorts with estimated lung cancer SMR>2.0, and studies conducted in Europe (UK manufacturing of gas masks), compared with other regions. Pooled SMRs were greater for cohorts exposed primarily to crocidolite (SMR=2.18, 95% CI: 1.40-3.37) or mixed asbestos (SMR=2.00, 95% CI:

1.41-2.84) compared to those exposed to chrysotile (SMR=1.40, 95% CI: 0.88-2.21), a finding similar to what others have observed for mesothelioma. However, this analysis⁴⁵¹ did not account for nonoccupational confounders other than age and was only restricted to highly-exposed women. Moreover, it was noted by Camargo et al.⁴⁵¹ that “[d]ifferences in the definitions of duration or latency of asbestos exposure measures prevented a proper evaluation of a dose-response relationship.” Another limitation of the Camargo study⁴⁵¹ is that only two studies had pathologic confirmation; estimates were attenuated in a sensitivity analysis in which it was assumed that 20% of the OvCa cases in each study were misclassified. The authors concluded that the meta-analysis supports the IARC conclusion that asbestos exposure is associated with increased OvCa risk despite the limitations mentioned above.

Reid et al.⁴⁵² conducted a meta-analysis of environmental and household studies totaling 14 cohort and two case-control studies and noted a statistically significant 75% excess OvCa risk in women who had been exposed to asbestos (effect size= 1.75 (95% CI: 1.45, 2.10)). Not surprisingly, the meta-analyses by Reid et al.⁴⁵² and Camargo et al.⁴⁵¹ have tremendous overlap with regard to the component studies they analyzed, including several studies discussed above.^{454,455,457,459,461,469,470} Thus, it makes sense that the summary ratios are similar to one another at 1.75⁴⁵² and 1.77 (1.37, 2.28),⁴⁵¹ respectively. However, in the meta-analysis by Reid et al.,⁴⁵² the association was attenuated and no longer statistically significant (effect size=1.29 (95% CI: 0.97, 1.73)) among studies that examined cancer incidence based upon pathologically confirmed cases.⁴⁵² This again suggests that the observed increased risks may be due to disease misclassification. Of note, the SMR was 40.9 and 64.0 for peritoneal mesothelioma and pleural mesothelioma, respectively, which is 8-13 times larger than that for OvCa (1.29 for studies that examined cancer incidence to 1.85 for all cohort studies). Moreover, a lack of consistency was observed; 4 of 14 cohort studies showed an excess rate for OvCa among women exposed to asbestos of the crocidolite type or a mixture of chrysotile and crocidolite. Of the remaining 10 studies, 5 failed to reach statistical significance and 5 reported rates similar to those of the reference population. No excess risk was reported among studies that examined incidence of OvCa where cases were ascertained from a cancer registry. It is also worth noting that loss to follow-up (>20%) was a significant issue

for several component studies, possibly contributing to an underestimation of person-years and overestimation of the SMR. Finally, no clear relationship was observed between standardized mortality or incidence ratios for mesothelioma and OvCa for the 9 cohort studies that examined both of these outcomes. Thus, even though exposure was sufficient to cause mesothelioma, no corresponding increased risk was observed for OvCa in this meta-analysis. Based on the body of accumulated data, Reid et al.⁴⁵² concluded that IARC's statement that asbestos exposure can cause OvCa⁴⁷¹ was premature and not fully supported by evidence.

A review paper by Slomovitz and colleagues similarly concluded that it is "imperative to question the IARC's assertion that asbestos has a clear causal inference to OvCa."⁴⁷² The authors⁴⁷² noted that connecting asbestos to OvCa is challenging due to the lack of randomized experimental trials. They point out flaws in the primary evidence (five cohort mortality studies^{404,405,410,412,426}) used by the IARC Working Group to conclude that asbestos as a cause of OvCa "was clearly established," noting the same concerns that were voiced earlier in this report. More specifically, Slomovitz et al.⁴⁷² mention that IARC's Working Group⁴⁷¹ reviewed several papers that revealed a non-significant risk of OvCa due to asbestos exposure, but they did not include them in the consensus opinion. This includes the cohort study of the national registry from Finland with more than 5,000 women that evaluated causes of OvCa and failed to uncover an association with asbestos, including among women exposed to the highest amounts.⁴⁶⁰ An additional case-control study from Norway that was not included in the consensus opinion studied women working in high asbestos-exposure printing jobs and also failed to find a statistically significant link to OvCa among pathologically confirmed cases.⁴⁷³ Slomovitz⁴⁷² also reiterates that "experimentally, there have not been reliable biological explanations *in vitro* or *in vivo* to explain the development of ovarian cancer due to asbestos." The authors⁴⁷² further state that "many of the epidemiologic studies of asbestos exposure suffer from additional and shared confounders—namely, the inability to review pathology and to distinguish between ovarian cancer and metastatic (pleural or peritoneal) malignant mesothelioma." From three studies that used pathologic review of specimens,^{454,458,463} there were 1,977 patients available and only 18 samples reviewed, 5 of which (28%) were misdiagnosed, a high rate of misclassification. The authors also

point out that none of the studies on which IARC based its judgment likely used “new immunohistochemical techniques,” as stated by IARC. Taken together, this assessment reiterates that the observed statistical associations between asbestos and OvCa are weak, inconsistent, and methodologically flawed.

In 2021, Nowak et al.⁴⁷⁴ from Germany asserted that occupational exposure to asbestos represents a “gynaecological occupational disease,” and comment on a law mandating to gynecologists that “suspected cases must be reported to the Statutory Accident Insurance carrier or the State Occupational Safety and Health Agency.” The intent of their manuscript is to provide background and rationale for this designation and recommendation, yet no compelling data is presented. With regard to evidence of the “pathomechanism of asbestos effects in humans, especially in the ovary”, the authors⁴⁷⁴ misleadingly cite the meta-analysis by Camargo et al.⁴⁵¹ as providing data that have “become more conclusive to the effect that ovarian cancer is also caused by asbestos.” As described earlier in this report, data from that meta-analysis⁴⁵¹ and its component studies should be interpreted with caution. Nowak et al.⁴⁷⁴ also erroneously state that the meta-analysis by Reid et al.⁴⁵² revealed a pooled effect estimate of 1.98 (95% CI: 1.32-2.97) among women with histological confirmation of OvCa. That is incorrect; as mentioned previously, Reid et al.⁴⁵² showed that the association was attenuated and no longer statistically significant (effect size=1.29 (95% CI: 0.97, 1.73)) among studies that examined OvCa incidence based upon pathologically confirmed cases. Nowak et al.⁴⁷⁴ admit that “the studies on which the newly recognised occupational disease ovarian carcinoma from asbestos exposure was based often had limitations such as poor histological validation or small patient numbers.” A pilot study supported by the German Statutory Accident Insurance (DGUV) was conducted to test the feasibility of a large-scale survey to “more precisely” evaluate the relationship between occupational asbestos exposure and OvCa. But, due to low participation rates, lack of precise data about asbestos exposure, and lack of medical record availability for most participants, the authors concluded that their nationwide survey of women with occupational exposure to asbestos “does not appear to make sense.” They⁴⁷⁴ conclude that “the responsible collection of asbestos history and reporting of suspected occupational diseases by each gynaecologist is of particular importance.”

In 2022, Vidican and colleagues⁴⁷⁵ published a multicenter case–case study of women recruited from four hospitals in Lyon, France, to assess the association between asbestos exposure and pathologist-confirmed histological subtypes of OvCa. Asbestos exposure was self-reported and defined as direct (occupational and environmental) and indirect (via parents, partners, and children), and an industrial hygienist assessed the probability and level of exposure. The prevalence of direct and indirect asbestos among the 254 enrolled patients was 13% (mean exposure duration 11 years) and 46%, respectively. High-grade serous carcinoma accounted for 73% of all OvCas and 82% and 72% of histological subtypes in women with direct and indirect asbestos exposure, respectively. After adjustment for family history of OvCa, no significant associations between asbestos exposure (direct and/or indirect) and high-grade serous carcinoma were detected. Study limitations include the low response rate (47%).

In 2022, Dalsgaard and colleagues⁴⁷⁶ focused on examining the risk of female cancer(s) in 6,024 former school children exposed to environmental asbestos in childhood while attending four schools in the neighborhood of a large asbestos cement plant in Denmark. The ‘exposed’ group were frequency-matched to a reference cohort on sex and five-year age intervals. Using Danish registries, linkage to historical employments, relatives’ employments, cancer status, and vital status was obtained. An increased incidence was reported for cancer of the corpus uteri (SIR 1.29, 95% CI 1.01-1.66) and malignant mesothelioma (SIR 7.26, 95% CI 3.26-16.15) compared to the reference cohort. Asbestos exposure was associated with a *lower risk* of OvCa or fallopian tube cancer overall (SIR 0.72, 95% CI: 0.52-1.01). The SIR for environmental asbestos exposure was 0.76 (95% CI: 0.54-1.08). In a study of 12,111 former school children from Aalborg, Denmark, Dalsgaard et al.⁴⁷⁷ again found the incidence of OvCa to be *lower* among the asbestos-exposed group (SIR 0.71, 95% CI: 0.50-0.99). Overall, my takeaway from these studies and other asbestos-related studies discussed in this report is that the epidemiological evidence does *not* support an increased risk of OvCa for those exposed to occupational or environmental asbestos.

Stoppa et al.⁴⁷⁸ recently sought to evaluate the spatial distribution of asbestos-related diseases by applying Bayesian shared models to the bivariate (two-disease) spatial distribution of ovarian and pleural cancer mortality by municipality in the Lombardy

Region of Italy between 2000-2018. They retrieved information on 10,462 deaths from OvCa, with SMRs ranging from 0 to 19.15. Three of the municipalities with high SMR of OvCa also had high pleural cancer rates. However, the majority of the high SMRs for OvCa were very unstable, as they were based on fewer than five expected cases. Wide uncertainty was also present when evaluating the risk of OvCa associated with pleural cancer in areas with low background risks of OvCa. The authors suggest this occurs because “the asbestos attributable fraction for ovarian cancer is low and population prevalence of asbestos exposure among women is not high.” Saito and colleagues⁴⁷⁹ also sought to compare mortality rates for lung and ovarian cancer between 2000 and 2017 in Brazilian municipalities where asbestos mines and asbestos cement plants had been operating compared to other municipalities. For OvCa, the SMRs were slightly higher before 1970 (1.39 vs. 1.26). Unfortunately, these studies^{478,479} must be interpreted with caution due to their ecologic design based on aggregate data (e.g., residence in certain municipalities) rather than individual level covariates. Such ecologic analyses can lead to spurious associations, and it is very likely that this is why such associations were detected in these studies^{478,479}.

In 2023, Turati and colleagues⁴⁸⁰ published an updated systematic review on OvCa among women occupationally exposed to asbestos, with an intent to explore the association with the time since first exposure and the duration of exposure. The authors⁴⁸⁰ noted that a few studies had been published since the time of the review by Camargo et al.⁴⁵¹, including a Chinese cohort of male asbestos miners,⁴⁸¹ an Italian cohort of workers from the asbestos cement industry⁴⁸², and an updated pooled analysis⁴⁶⁵ of Italian asbestos cement workers.⁴⁸³ Two studies that were included in the systematic review by Camargo and colleagues⁴⁵¹ were excluded from the current analysis;⁴⁸⁰ one because “asbestos exposure was very limited and confined to a small proportion of workers⁴⁸⁴, and the second⁴⁸⁵ because data was already included in the study by Pira⁴⁶⁴. Eighteen publications representing 20 populations, including a pooled analysis of 21 cohorts by Luberto et al.⁴⁶⁵ and an analysis from 2020 by Fazzo et al.⁴⁸² were included. The manuscript by Luberto et al.⁴⁶⁵ rather than one by Magnani et al.⁴⁸³ was selected because the latter also included cohorts of glassworkers and women with household exposure. The pooled SMR was 1.79 (95% CI: 1.38–2.31) based on 144 OvCa deaths/cases, and

significant heterogeneity was reported between studies ($I^2 = 42\%$). Higher SMRs were estimated in Europe and Asia, while OvCa mortality was *lower* (e.g., the number of observed deaths from OvCa among those exposed to asbestos was lower than expected) in the US and Australia (SMR: 0.92, 95% CI: 0.54-1.59). The authors⁴⁸⁰ indicated this may be due to an earlier time of exposure and higher use of amphiboles in Europe compared to North America. In the pooled analysis, a significant proportion of weight was given to studies by Harding et al.⁴⁸⁶ (8.98%), Pira et al.^{464,487} (8.92%), and Luberto et al.⁴⁶⁵ (9.28%); each of these studies were likely affected by misclassification bias since they did not confirm pathology to distinguish between ovarian or peritoneal cancer and metastatic (pleural or peritoneal) malignant mesothelioma. The authors state that their “findings from an analysis assessing misclassification of mesothelioma as ovarian cancer suggest that misclassification cannot be excluded; however, misclassification alone cannot explain the observed excesses of both mesothelioma and ovarian cancer.” They⁴⁸⁰ also state that “the effect of duration and latency could not be completely disentangled, since no multi-variate analysis was available for time-related variables.” Based on these limitations, I disagree with their conclusion that “the excess risk of ovarian cancer should be added to the overall burden of morbidity and mortality caused by occupational exposure to asbestos.”

Collectively, these recent publications^{474-476,478-480} on the purported asbestos-OvCa association have not produced any compelling data to support causality. The observed statistical associations between asbestos and OvCa remain weak, inconsistent, and are based on methodologically flawed designs and analyses. Furthermore, studies assessing asbestos and OvCa risk are largely addressing significant occupational or environmental exposure to asbestos, which is very different than what is being alleged in this litigation.

Several studies have evaluated ovarian tissue for the presence of asbestos. Asbestos fibers have been reported in ovaries of women exposed to asbestos in the Norwegian pulp and paper industry⁴⁷³ and also among women whose household contacts worked with asbestos.⁴⁸⁸ In a study by Langseth et al.,⁴⁷³ the case group included ovarian tissue specimens from 46 women diagnosed with OvCa in the period 1953-2000 who had worked in at least one pulp and paper mill between 1920 and 1993. Normal ovarian tissue

specimens from two control groups without an occupational history of pulp and paper work were selected from the Cancer Registry database for comparison. Tissue blocks were digested, and transmission electron microscopy (TEM) was used to identify asbestos fibers. Asbestos fibers were found in normal ovarian tissue from two subjects in the case group, while no fibers were found in the control groups. One asbestos positive case had worked as a paper sorter/packer and the other as a chlorine plant worker, and both were potentially exposed secondarily to asbestos from family members working as insulators.

In a study of ovaries from 13 women with household contact with men who had documented asbestos exposure and 17 women undergoing incidental oophorectomy, ovarian tissue was also examined by electron microscopy.⁴⁸⁸ Asbestos fibers of chrysotile and crocidolite types were detected in 9 of 13 women with household asbestos exposure (69.2%) and in 6 of 17 women who gave no exposure history (35%). The authors noted that “the chrysotile and crocidolite types of asbestos we detected are more indicative of background and/or occupational exposure.”⁴⁸⁸

Taken together, a causal link between asbestos exposure and OvCa *cannot* be firmly established from the epidemiologic literature, both because the studies included small numbers of women and because of the likelihood of disease misclassification (i.e., pleural and peritoneal mesothelioma, asbestos-related diseases, have often been misdiagnosed as epithelial OvCa on death certificates). As noted above, analyses restricted to studies of pathologically confirmed OvCa cases did *not* find statistically significant associations.⁴⁵² Moreover, known confounders of an asbestos-OvCa association were not adjusted for in most of the aforementioned studies. Additionally, as noted by IARC⁴⁷¹, there was “wide variation” in approaches taken for exposure assessment, with some studies making “no attempt to assess exposure beyond documenting employment of study participants in a trade or industry with potential for occupational exposure to asbestos,” while others “used surrogate indices of exposure such as duration of employment or self-reported intensity of exposure, or stratified subjects’ exposure by job title.” Only a few studies obtained direct measurements of asbestos dust levels in the air using various measures, some of which were imprecise. Furthermore, the types, fiber sizes and levels of asbestos exposure are known to differ

among industries and over time and were not accounted for. There are also differences in how studies dealt with the issue of latency (i.e., time since first occupational exposure to asbestos). While earlier studies estimated person-years from first exposure, which may dilute observed risk by including many years of low risk, others only estimated person-years for a certain time period *after* the first exposure, typically 20 years.

It is critical to consider that the aforementioned studies are based on inhalation, and *not* perineal application. If indeed talc contained asbestos, a greater burden of any inhaled asbestos should be seen in the lungs due to direct exposure (rather than the ovaries, which purportedly would be indirectly exposed, if at all). To date, however, there are no compelling studies that have shown that mesothelioma or lung cancer risk is higher among talc users. In fact, Lynch et al.⁴⁸⁹ recently conducted a systematic review to evaluate the potential pulmonary carcinogenicity of inhaled talc among humans. Their review adhered to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and leveraged frameworks from the US Institute of Medicine (IOM) and the US Environmental Protection Agency (EPA). Lynch et al.⁴⁸⁹ identified primary studies that evaluated talc exposure and pulmonary cancer risks in humans (n=19) and animals (n=3). After integration of several lines of evidence, the authors⁴⁸⁹ concluded there was “suggestive evidence of no association between inhaled talc and lung cancer and pleural mesothelioma at human-relevant exposure levels.” Mundt and colleagues⁴⁹⁰ also recently reviewed findings of epidemiological studies on the association between lung cancer risk and occupational exposure to each of three different ‘inhaled’ particles classified as “poorly soluble low toxicity” or “PSLT” particles): carbon black, talc and taconite. They⁴⁹⁰ concluded that epidemiological evidence indicates that at very high occupational exposure levels at which non-malignant respiratory diseases including pneumoconiosis and talcosis are observed, lung cancer risks do not appear elevated.

Finally, studies that have evaluated talc miners and millers (who would have had exposure to asbestos if it were present in talc) have *not* reported any increased incidence of mesothelioma or lung cancer attributable to talc exposure in the mines or mills.^{5,464,491-493} For example, updated analyses of data from Italian cohorts of cosmetic miners/millers continue to fail to support an association between inhalation of cosmetic talc and an increased risk of mesothelioma^{492,493} or lung cancer⁴⁹³. Additionally, a systematic review

by Lewis et al.⁴⁹⁴ that evaluated occupational exposure to cosmetic talc among barbers, hairdressers, and cosmetologists, did not show an increased risk for mesothelioma. Findings for the occupations were null and if statistically significant, showed an inverse relationship, indicative of a protective effect of these occupations on mesothelioma risk. Furthermore, a recent quantitative weight of evidence analysis was performed in accordance with Hill's guidelines⁴⁹⁵ to assess epidemiological, toxicological, and exposure studies related to cosmetic talc and the risk of mesothelioma. The overall probability of causality for cosmetic talc and mesothelioma was reported to be low and approximately 1.29% (range: 0.73%–3.96%), supporting the conclusion that cosmetic talc is not related to the development of mesothelioma. Despite the suggestion that asbestos or talc fibers could migrate through the diaphragm, access the lymphatic system, and then reach the peritoneal cavity,⁴⁷⁹ I have also yet to identify a mechanistic study that demonstrates that inhaled talc particles can reach the ovaries and promote ovarian carcinogenesis.

In summary, studies in humans and animals and limited *in vitro* studies of asbestos and ovarian cells^{473-476,478-480,488,496} have *not* provided compelling support for the theory that asbestos exposure can cause OvCa. I disagree with the IARC working group's⁴⁴⁹ conclusion that a causal association between exposure to asbestos and OvCa has been established based on the assessment of a) five mortality studies of women with heavy occupational exposure to asbestos;^{454,455,458,461,463} b) studies showing that women and girls with environmental exposure to asbestos had positive yet statistically insignificant increases in OvCa incidence and mortality;^{456,457,462} and c) modest support from findings of non-significant associations in two case-control studies.^{460,466} It is important to understand that even though asbestos is a carcinogen, questions of carcinogenicity need to be addressed tissue by tissue, and *a causal link with OvCa has not been demonstrated* based on the collective body of evidence available at this time.

II. OVERVIEW OF METHODS AND ANALYSIS OF TALC AND OVCA

Presented in the remainder of this report is my expert opinion on claims alleging that talc-based products manufactured by a subsidiary of Johnson & Johnson have caused women to develop ovarian cancer (OvCa). As an epidemiologist with expertise in molecular biology, genetics and gynecologic oncology, I have the educational

background, training, and experience to assess these claims using a methodologically sound and balanced approach. Importantly, as a long-time patient advocate and former genetic counselor, I am committed to identifying factors (especially those that are modifiable) that could diminish an individual's cancer risk so that strategies can be developed to prevent disease among those who may be at risk of developing it. Women are not benefited, however, by faulty science. Given the knowledge available at this time, I believe that the plaintiffs' hypotheses as to causation are flawed due to numerous methodological, statistical and biological issues and that reliable and robust scientific evidence is *lacking* to support the plaintiffs' contention that talc causes OvCa.

To form this opinion, I have conducted a systematic review of epidemiologic studies, lab-based molecular and toxicology studies, and other relevant studies discussed herein. In conducting my systematic review, I searched for studies using the PubMed database and search terms including, "ovarian cancer", "talc", and "epidemiology". I also reviewed studies that were noted, discussed, or cited in the publications that I identified. Additionally, I reviewed studies cited by Plaintiff's experts, as discussed further below. Each study was evaluated based on the study design, methods, statistical analysis, results, and conclusions. I also considered additional studies and data related to other risk factors for ovarian cancer that are discussed in the scientific literature, and I analyzed public and published statements from national and international agencies that study and research cancer. Subsequent to conducting my systematic review, I analyzed the studies and literature and provided my judgment as to how the study findings align with scientific knowledge and established criteria for assessing causation. In conjunction with this discussion, I refer specifically to the Bradford Hill guidelines, including strength of association, consistency, specificity, temporality, biologic gradient, plausibility, coherence, experiment, and analogy. I also discuss those factors thought to be most important in assessing causality, and evaluate the relevant scientific literature with these guidelines and factors in mind in order to reach a causal conclusion.

A. Overview of epidemiologic study designs

I first reviewed scientific literature comprised of peer-reviewed epidemiologic studies and abstracts related to talc, OvCa, gynecologic conditions and related scientific issues that experts should be relying on when evaluating the postulated talc-OvCa

association. (In some studies, talc exposure was the primary focus, but in others, talc was not the primary focus.) Epidemiologic studies generally fall into two broad categories: observational studies and experimental studies. In observational studies, the investigators collect, record and analyze data on participants *without* controlling study conditions or exposure status. By contrast, in experimental studies, investigators intervene and control study conditions *and* exposure status. Because it is often unethical to intervene and expose individuals to an agent under investigation, most human epidemiologic studies, including those related to the alleged talc-OvCa association, are observational in nature. Observational studies can be descriptive or analytic. Whereas descriptive studies focus on characterizing health outcomes by person, place or time and have no specified *a priori* hypotheses to test, analytic studies test *a priori* hypotheses about exposure-disease associations.

Two main types of analytic observational studies have been conducted to evaluate the talc-OvCa risk hypothesis: “case-control” studies and “prospective cohort” studies. A case-control study compares two groups of people: those with the disease or outcome of interest (cases), and a similar group who do not have the outcome (controls). Researchers can then look back in time (retrospectively) and study the history of exposures of the people in each group to learn what factors or exposures may be associated with the disease. In a prospective cohort study, a group of people who are disease-free at baseline are followed forward in time to determine the incidence of particular disease(s). Because exposure (or lack thereof) to certain substances is known at baseline and precedes the outcome, cohort studies are typically considered to provide stronger evidence for causation than case-control studies. All of the studies that have reported associations (albeit weak) between talc and OvCa risk have been case-control studies, whereas no evidence for a statistically significant association with OvCa risk overall has been observed in prospective cohort studies. Since 2000, a total of four studies have prospectively evaluated the talc-OvCa association,^{31,497-499} and a pooled analysis of updated data from four prospective cohort studies has recently been performed.⁵⁰⁰ The prospective studies by Gertig et al.⁴⁹⁷ and Gates et al.³¹ involve the same population (the Nurse’s Health Study (NHS) I Cohort), but the study by Gates et al.³¹ includes 10 additional years of follow-up. *None* of the aforementioned prospective

cohort studies^{31,497-499} supports the plaintiffs' claims that talc causes OvCa. Furthermore, the recent pooled analysis of data from women in four US cohorts (N=252,745 women and 2,168 OvCa cases) published in JAMA does *not* show a statistically significant association between use of powder in the genital area and incident OvCa.⁵⁰⁰

Epidemiologic study designs and their ranking for assessing causality

In his textbook on epidemiologic designs and methods,⁵⁰¹ Dr. William A. Oleckno, a well-known epidemiologist and scholar, ranked the common types of epidemiologic studies in *descending* order of the degree to which similar findings of a statistical association (based on cumulative information from multiple studies) are likely to demonstrate a causal association. This ranking is based on the probability of finding unrecognized *bias*, *confounding* or other errors within certain study designs, and assumes that studies have been planned appropriately and conducted to reduce the number of errors. Bias or systematic error can be defined as a difference between an observed value and the true value due to causes other than sampling variability. Confounding refers to a situation in which a variable that is associated with both the exposure and outcome is responsible for the entirety or part of the observed statistical association between the exposure and outcome.

Experimental studies (and specifically randomized controlled trials) were ranked as the most likely to have findings that represent causal associations, followed by prospective cohort studies, retrospective cohort studies, case-control studies and other types of analytic and descriptive observational studies.⁵⁰¹ Thus, within analytic observational studies, prospective cohort studies are ranked *higher* than case-control studies for being likely to demonstrate a causal association *if* one exists. This hierarchy of evidence with respect to human studies is widely recognized by epidemiologists.

B. Case-control studies are more prone to bias than prospective cohort studies

There are two main types of case-control studies, hospital-based and population-based, and both are more prone to bias than the prospective cohort design. In a hospital-based case-control study, subjects are selected among patients admitted to hospitals or clinical facilities. "Cases" are patients with the outcome of interest, whereas hospital "controls" are patients admitted to the same clinical facility as the cases but *without* the

study outcome. On the other hand, in a population-based case-control study, cases and controls are selected from a representative sample of a defined population. In each of these study designs, bias can arise from numerous sources, including factors involved in the choice or recruitment of a study population and factors involved in the definition and measurement of study variables. The inverse of bias is validity, which is a desired attribute.

Two forms of bias that can occur frequently in case-control studies are *selection bias* and *information bias*. Selection bias is a systematic error that results from the way in which subjects are selected or retained in a study. This bias can occur when the characteristics of the subjects selected for a study systematically differ from those in the source population, or when the study and comparison groups are selected from different populations. Information bias is a type of systematic error that occurs during the data collection phase of a study due to measurement flaws. Such flaws can result in misclassification of subjects with regard to exposure or outcome status. Two types of information bias are recall bias and interviewer bias. Recall bias occurs when the accuracy of recollection of exposure or use differs between cases and controls such that a case may be more likely to (correctly) recall the past use of an agent than a control (who might forget) or a case may be more likely than a control to *incorrectly* report the use of an agent that was never used. In case-control studies of talc and OvCa, it is very possible that recall bias may lead to an overestimation of the true association because cases are already aware of their diagnosis when reporting talc use. Interviewer bias can occur when the individual asking about the exposure is aware of disease status (case versus control).

C. Talcum Powder and OvCa: A Comprehensive Review of the Literature

Talc is a naturally occurring mineral composed of magnesium silicate that absorbs water and has many uses in cosmetics, other personal care products, tablets, food and gum. While some epidemiologic studies summarized below have reported a positive association between talc use and OvCa risk, other, more robust studies have not. Furthermore, mechanistic, pathology and animal studies do not provide supportive evidence for the hypothesized carcinogenicity of talc on the ovarian epithelium. Below is a summary of some heavily cited studies that evaluated the purported talc-OvCa risk

association in chronological order of their publication year; case-control studies are addressed first and are followed by prospective cohort studies. After individual studies are described, findings from several older meta-analyses,^{414,502,503} one pooled analysis of case-control studies,⁵⁰⁴ three recent systematic reviews and meta-analyses,^{413,416,505} and one recent pooled analysis of prospective cohort studies⁵⁰⁰ are summarized and compared (**Table 2**).

i. Retrospective Case-control Studies

In 1982, Cramer et al.⁵⁰⁶ first evaluated genital exposure to talc in 215 White females with epithelial OvCa and 215 controls from the general population matched to cases on age, race and residence (**Table 1**). Of concern, 33% of potential controls refused to participate, possibly contributing to selection bias. Ninety-two (42.8%) of the OvCa cases used talc regularly as a dusting powder on the perineum or on sanitary napkins compared to 28.4% (n=61) of controls. After adjustment for parity and menopausal status, the authors found an RR of 1.92 (95% CI=1.27-2.89, P<0.003) for users with perineal exposure to talc compared to non-users, and statistically insignificant RR of 1.55 (95% CI=0.98, 2.47) and 1.19 (95% CI: 0.69, 2.05) for talc use on sanitary napkins and diaphragms, respectively. Thirty-two women who used talc on the perineum *and* on sanitary napkins had an adjusted RR of 3.28 (95% CI=1.68-6.42) compared to women with neither route of exposure, though this estimate should be interpreted with caution due to the small number of women exposed.

In 1983, Hartge and colleagues⁵⁰⁷ reported on a hospital-based case-control study of 135 cases and 171 controls conducted between 1974 and 1977 in the Washington, DC area. Talc applied to the perineum was not associated with OvCa risk (RR 2.50, 95% CI: 0.70, 10.0). Similarly, talc dusted on diaphragms was not risk-associated (RR=0.80) (**Table 1**). In a hospital-based study of personal and environmental characteristics related to OvCa risk among women in the San Francisco area, Whittemore and colleagues³³⁷ evaluated 188 women diagnosed with OvCa between 1983 and 1985 and 539 controls (comprising 280 hospitalized women without cancer and 259 identified through random-digit dialing). Talc use was not associated with OvCa risk, regardless of the mode of application (perineum, sanitary napkin, diaphragm) (**Table 1**). Moreover, dose-response trends were not observed for frequency or duration of use (**Table 3**).

Booth et al.¹³⁶ conducted a hospital-based case-control study in London and Oxford between 1978 and 1983, and compared the distribution of known and putative risk factors among 235 OvCa cases and 451 controls. Women who reported talc use daily (RR=1.3 (95% CI: 0.8-1.9)) had a lower risk for OvCa than those reporting weekly use (RR of 2.0 (95% CI: 1.3-3.4)). Thus, there was *not* a consistent trend of increasing risk with increased frequency of talc use after adjustment for age and social class (**Table 3**). Additionally, the women were not asked how long they had been using talc. The authors pointed out that due to their symptoms or disease-related pelvic examinations, the frequency of current use may not have been representative of their past use.

Harlow and Weiss⁵⁰⁸ evaluated the influence of hygienic powders on the risk of serous and mucinous borderline ovarian tumors among 116 cases from western Washington state and 158 women identified through random digit dialing. Of note, approximately 30% of eligible cases and controls were not included due to refusals or other reasons for non-participation. While perineal application of baby powder and cornstarch (and users of talc for diaphragm storage) were *not* associated with risk (**Table 1**), women who used deodorizing powders alone or in combination with other talc-containing powders had 2.8 times the risk (95% CI: 1.1-11.7) of developing a borderline ovarian tumor compared with women who had not used perineal talc. The authors⁵⁰⁸ concluded that the risk of ovarian tumors in women who apply deodorizing powder to the perineum may not relate to talc per se, but rather to asbestos contamination and/or a substance used for deodorization such as free and bound silicas.

In a study by Chen et al. that was conducted in Beijing,¹⁷⁰ 112 pathologically confirmed OvCa cases and 224 age-matched community controls were evaluated for reproductive, medical, familial and selected lifestyle factors such as talc use. Women with a history of >3 months of talc dusting to the lower abdomen and perineum had an increased risk of OvCa that was not statistically significant (RR=3.9, 95% CI: 0.9-10.63).

Harlow et al.⁵⁰⁹ interviewed 235 White women diagnosed with OvCa between 1984 and 1987 at 10 Boston metropolitan area hospitals and 239 population-based controls of similar race, age and residence. Forty-nine percent of cases and 39% of controls reported exposure to talc via direct application to the perineum or to underwear, sanitary napkins or diaphragms. Application to the perineum, sanitary napkin and diaphragm all yielded

ORs ranging from 1.1 to 1.5, none of which was statistically significant (**Table 1**). The greatest OvCa risk associated with perineal talc use was observed in the subgroup of women estimated to have had at least 30 applications of talc per month (OR 1.8 (95% CI: 1.1-3.0)); however, this exposure was only found in 14% of women with OvCa and there was *not* a clear trend with frequency or duration of use (**Table 3**) despite what others reported.⁵⁰⁵ The authors also evaluated whether OvCa risk was affected by the time talc-containing products were manufactured; women who only used talcum powder before 1960 had a higher risk (OR=1.7, 95% CI: 1.1-2.7) than those who used talc after 1960 (OR= 1.1, 95% CI:0.6-2.1). Of note, there may have been possible under- or over-reporting of talc use since researchers only interviewed 69% of eligible cases and 81% of eligible controls.

Rosenblatt et al.⁴⁶⁷ conducted a small hospital-based case-control study of the association between genital powder use and OvCa risk at the Johns Hopkins Hospital in Baltimore, Maryland among 77 incident OvCa cases ascertained between 1981 and 1985 and 46 age- and race-matched controls who were inpatients who self-reported that they did not have gynecologic or malignant conditions. No statistically significant increase in OvCa risk was observed with: genital powder use after adjustment for parity (OR: 1.0, 95% CI: 0.2-4.0); diaphragm use with powder after adjustment for parity and education (OR: 3.0, 95% CI: 0.8-10.8); genital bath talc exposure (OR: 1.7, 95% CI: 0.7-3.9); or length of exposure (>37.4 years vs <37.4 years) (OR: 2.4, 95% CI: 1.0-5.8).

In a hospital-based case-control study of OvCa conducted in Greece between 1989 and 1991, 189 women with histologically confirmed malignant epithelial tumors of the ovary were compared with 200 hospital-based controls.⁵¹⁰ In addition to evaluating whether hair dye, tranquilizing and hypnotic drugs, and analgesics were associated with OvCa risk, the authors also evaluated whether perineal application of talc was risk-associated. No such association between talc and OvCa risk was identified (adjusted RR 1.05; CI: 0.28, 3.98), but the authors noted that frequency of reporting talc use was low in the study population.

In 1995, Cramer et al.⁵¹¹ published on the association between prior hysterectomy or tubal ligation and OvCa risk by combining two previously conducted case-control studies (Cramer et al. (1982)⁵⁰⁶ and Harlow et al. (1992), discussed above) totaling 450

women with histologically verified epithelial OvCa and 454 age-matched women from the general population who had available data on prior pelvic surgery. A protective effect of prior hysterectomy or tubal ligation was more evident (but not statistically significant) among women who had the surgery 20 or more years previously (OR = 0.6, 95% CI, 0.3 to 1.1), women who had not used genital talc (OR = 0.6, 95% CI, 0.4 to 1.0), and women with mucinous tumors of the ovary (OR = 0.3, 95% CI, 0.1 to 1.0). The risk of OvCa associated with pelvic surgery among talc users was not statistically significant (OR=1.1, 95% CI: 0.6-2.1). The authors speculated that mechanisms for an association between prior pelvic surgery and lower ovarian cancer risk may be mediated through absent or reduced uterine growth factors that reach the ovaries through the uteroovarian circulation.

Purdie et al.¹⁴⁰ conducted a case-control study involving personal interviews with 824 women diagnosed with epithelial OvCa in three Australian states (Queensland, New South Wales and Victoria) between August 1990 and December 1993 and 860 controls drawn at random from the electoral roll, stratified by age and geographic region. Findings confirmed the reduced risk of OvCa associated with increasing parity and duration of use of oral contraceptive pills, hysterectomy and tubal ligation. Of other risk factors considered, high body mass index, family history of OvCa and use of talc in the abdominal or perineal region (RR=1.27, 95% CI: 1.04, 1.54) were positively associated with OvCa risk.

A case-control study published in 1997 by Cook et al.⁵¹² evaluated the risk of OvCa associated with genital exposure to various forms of powder. Cases included all women aged 20-79 years in three counties of western Washington diagnosed with borderline or invasive OvCa from 1986 through 1988. Approximately 64% of eligible cases were interviewed, for a total of 313 women. A sample of similarly aged women who lived in these counties, identified by random digit dialing, served as controls; only 68% of controls participated, for a total of 422 women. An increased OvCa risk was noted for women with a history of perineal dusting (adjusted RR=1.6 (95% CI:1.1, 2.3)) or use of genital deodorant spray (RR=1.9, 95% CI:1.1, 3.1) (**Table 1**). Storing a diaphragm in powder 1.00 (95% CI: 0.60, 1.70) or powdering sanitary napkins 0.90 (95 CI: 0.50,1.62) were not related to OvCa risk (**Table 1**). Furthermore, higher cumulative lifetime months of use and/or lifetime applications were *not* significantly associated with OvCa risk (**Table 3**).

In a case-control study of 450 borderline and invasive OvCa cases and 564 population-based controls published in 1997, Chang and Risch⁵¹³ found that exposure to talc applied directly to the perineum was statistically significantly associated with OvCa risk among Canadian women (OR=1.42, 95% CI: 1.08-1.86). No statistically significant association was found for talc use on sanitary napkins. Furthermore, OvCa risk did not increase based on greater duration or frequency of exposure (**Table 3**).

Also in 1997, Green et al.²⁷¹ (who evaluated the same study population as Purdie et al.¹⁴⁰) studied the effect of tubal sterilization or hysterectomy on a woman's risk of developing OvCa in a case-control study in eastern Australia. To evaluate the hypothesis that tubal occlusion may prevent entry of foreign agents to the peritoneal cavity through the fallopian tubes, they assessed exposure to vaginal sprays and douches, contraceptive foams and talcum powder. Duration of exposure was calculated from age at first use to earliest age of pelvic surgery, if applicable. Associations between painful or heavy menstrual periods and OvCa were also assessed to identify women who may have experienced more pronounced retrograde menstruation, with the thought being that tubal occlusion may prevent retrograde menstruation. Women who had heavy periods before hysterectomy tended to have a lower OvCa risk after surgery than women who reported average or lighter periods. This observation was not seen for tubal sterilization. Of 824 women with incident OvCa and 855 controls, 104 cases (13%) and 194 controls (23%) reported tubal sterilization. OvCa was found to be reduced by 39% after tubal sterilization and by 36% after hysterectomy. Only perineal talc use was associated with a 30% increased risk (95% CI: 1.10, 1.54); talc exposure from vaginal sprays, foams, condoms, and diaphragms were not associated with OvCa (**Table 1**).

In a case-control study of French Canadian women in Montreal between 1995-1996 by Godard et al.,⁵¹⁴ risk factors were compared between familial and sporadic OvCa cases. One hundred seventy women 20 to 84 years old with histologically confirmed diagnoses of primary ovarian carcinomas or borderline tumors were interviewed regarding their reproductive, family, and medical histories, as were 170 randomly selected population control subjects, frequency-matched to the case patients according to age and ethnic group. Of note, the response rate was only ~23% among controls compared to 87% among cases, possibly biasing results toward a positive association because of the

potential for recall bias among cases. The main factors contributing to OvCa risk were: family history of breast or ovarian cancer, a late age at use of oral contraceptives (a protective effect) and a late age at last childbirth (a protective effect for familial case patients only). An increased but statistically insignificant risk for OvCa was observed among women with a history of talc use (**Table 1**); this was observed for both the familial and sporadic groups.

In 1999, Cramer performed another study of genital talc exposure and risk of OvCa⁵¹⁵ in a population-based study of 563 women newly diagnosed with OvCa in eastern Massachusetts or New Hampshire between 1992 and 1997, and 523 controls. Cases were more likely than controls (45% vs 36%) to use talc or body powder in some manner, with the excess confined to patients who used talc on the perineum directly or as dusting powder to underwear or sanitary napkins. Compared to women who never used body powder or only used it on non-genital areas, the OR associated with genital exposure to talc was 1.60 (95% CI: 1.18-2.15) after adjustment for age, study location, parity, oral contraception use, body mass index and family history of breast or OvCa (**Table 1**). Exposure prior to (rather than after) the first livebirth appeared to have more of an association, and the association was most apparent for women with invasive serous cancers and least apparent for those with mucinous tumors.

Also in 1999, Wong et al.⁵¹⁶ published on the evaluation of perineal talc exposure and subsequent OvCa among women with OvCa versus women without OvCa who had a similar distribution of age as the cases. Talc powder use was reported among 221 of 462 cases (47.8%) in the study population and 311 of 693 patients (44.9%) in the control population, with an adjusted OR= 0.92 (95% CI:0.24-3.62). Compared to non-users, a significant association between duration of talc use and development of OvCa was not demonstrable among those with 1-9 years of use (OR:0.90, 95% CI 0.60-1.50), those with 10-19 years of use (OR: 1.40, 95% CI: 0.90-2.20) or those with more than 20 years of use (OR: 0.90, 95% CI: 0.60-1.20) (**Table 3**). To eliminate the possible confounding variable of surgery, the authors omitted 135 patients who had undergone hysterectomy within five years of the diagnosis of OvCa. Within this subgroup of patients, tubal ligation or hysterectomy among talc users did not have an increased risk of OvCa (OR:0.9, 95% CI:0.4,2.2), even with prolonged exposure. Of note, nearly half of the control population

used for this study comprised women with a history of colorectal cancer; such individuals are not appropriate to include since they may be at higher than average risk for OvCa compared to others in the general population. Also of concern, about 15% of subjects did not answer questions related to talc use or failed to provide details about such use.

Ness et al.⁵¹⁷ examined tubal ligation, hysterectomy and perineal talc use in the context of the hypothesis that inflammation may play a role in OvCa risk, among 767 newly diagnosed cases of epithelial OvCa 20-69 years of age and 1,367 community controls. The authors proposed that “ovulation entails ovarian epithelial inflammation, talc, endometriosis, cysts, and hyperthyroidism may be associated with inflammatory responses of the ovarian epithelium, and gynecologic surgery may preclude irritants from reaching the ovaries via ascension from the lower genital tract.” They found that reproductive and contraceptive factors that suppress ovulation (including gravidity, breast feeding and oral contraception) reduced OvCa risk, while environmental factors and medical conditions (including talc use (RR=1.50, 95% CI: 1.10, 2.05)) (**Table 1**), endometriosis and ovarian cysts increased risk. No dose response was seen with frequency or duration of talc use (**Table 3**).

As mentioned previously, Langseth and Kjaerheim⁴⁶⁶ evaluated occupational exposure to asbestos and occupational exposure to talc in a cohort of pulp and paper workers. They also evaluated hygienic talc use (on sanitary napkins, underwear or diapers) in this study. As shown in **Table 1**, exposure to hygienic talc was *not* associated with a statistically significant increased risk among the small subset of participants (19 cases, 95 controls) who answered questions on this topic (OR:1.15, 95% CI:0.41,3.21). Thus, the results of this study do not support an association between exposure to asbestos, talc, and total dust and OvCa among Norwegian pulp and paper workers.

Mills et al.⁵¹⁸ conducted a population-based case-control study of perineal talc exposure and OvCa risk in 22 counties of Central California that comprise the reporting area for two regional cancer registries. Frequency and duration of talc use and specific years of talc use were assessed by performing telephone interviews for 256 cases diagnosed between 2000-2001 and 1,122 controls frequency-matched on age and ethnicity. Ever use of talc was associated with an increased OvCa risk (OR=1.37 (95% CI: 1.02-1.85)) compared to never use (**Table 1**). Risk was highest among women with

serous invasive tumors (OR=1.77, 95% CI: 1.12-2.81) (**Table 4**). No clear dose response was identified (**Table 3**).

In 2005, Cramer et al.⁵¹⁹ proposed that the talc-OvCa association may be explained by an immune-mediated mechanism. In this study, talc was not associated with OvCa risk (OR=1.16, 95% CI: 0.90-1.49). Because talc use correlated with decreased levels of anti-MUC1 antibodies in the blood, it was suggested that exposure of the lower genital tract to talc may promote irritation and inflammation and subsequent reduction of anti-MUC1 antibodies and protective immunity. However, no experiments were performed to test this hypothesis. Taken together, because evidence is lacking to support anti-MUC1 antibodies as playing a role in immune surveillance of OvCa, the aforementioned study should be considered purely speculative.

Gates et al.⁵²⁰ analyzed interactions between talc use and genes in detoxification pathways [glutathione S-transferase M1 (GSTM1), glutathione S-transferase T1 (GSTT1), and N-acetyltransferase 2 (NAT2)] to evaluate whether the talc-OvCa association is modified by variants of genes potentially involved in the response to talc. Their analysis included 1,175 cases and 1,202 controls from a New England-based case-control study and 210 cases and 600 controls from the prospective Nurses' Health Study, a study population that overlaps with others mentioned in this report.^{31,497,504,521} Regular talc use was associated with increased OvCa risk in the combined population (RR 1.36, 95% CI, 1.14-1.63; P(trend) < 0.001) and the talc-OvCa association varied by GSTT1 genotype and combined GSTM1/GSTT1 genotype, with the authors suggesting that women with certain genetic variants may have a higher risk of OvCa associated with genital talc use. However, no biological mechanism was suggested or tested to explain why higher levels of GSTM1 and lower levels of GST1 increase interaction with talc.

Merritt 2008³⁰⁷ analyzed the potential role of chronic local ovarian inflammation in the development of the major histologic subtypes of epithelial OvCa by leveraging an Australia-wide case-control study that included 1,576 women with invasive and LMP ovarian tumors and 1,509 population-based controls. The authors identified a marginally statistically significant increase in OvCa risk associated with use of talc in the pelvic area (adjusted OR 1.17, 95% CI: 1.01-1.36) that was strongest for women with the serous and endometrioid subtypes, though the latter was not statistically significant (adjusted OR

1.21, 95% CI 1.03-1.44 and 1.18, 95% CI 0.81-1.70, respectively) (**Tables 1 and 4**). Other factors potentially associated with ovarian inflammation (pelvic inflammatory disease, human papilloma virus infection) were not associated with OvCa risk. Regular use of aspirin and other NSAIDS was inversely associated with risk of LMP mucinous ovarian tumors only. The authors concluded that chronic inflammation does not appear to play a major role in OvCa development.

Moorman 2009⁵²² utilized data from a North Carolina case-control study (1999-2008) to evaluate risk factors for OvCa among African American/Black (143 cases, 189 controls) and White (943 cases, 868 controls) women. Tubal ligation and family history of breast cancer or OvCa showed stronger associations among Blacks, while younger age at menarche was associated with risk only in White women. Talc use was not associated with OvCa risk among Blacks (1.19 (95% CI: 0.68, 2.09)) or Whites (1.04 (95% CI: 0.82,1.33)).

To investigate factors that may increase inflammation and OvCa risk, Wu et al.⁵²³ examined the role of talc, history of endometriosis and use of NSAIDS in a population-based case-control study in Los Angeles County. The study included 609 women with newly diagnosed OvCa and 688 population-based controls recruited from neighborhoods. Participants were interviewed in person and asked about frequency and duration of talc use. Ever use in the perineal area was associated with an increased OvCa risk (RR=1.53, 95% CI: 1.13-2.09) (**Table 1**). Risk patterns by frequency and duration of talc use generally showed trends of increased risk with increasing frequency of use and longer durations, but a statistically significant association was only present among the 67 cases and 45 controls with the highest frequency and duration of talc use (RR=2.08, 95% CI: 1.34,3.23) (**Table 3**). Similarly, when considering cumulative dose, a statistically significant association was only observed among those with >52,000 applications (RR=1.99, 95% CI:1.34-2.96). Compared with women who were non-users of talc and who did not have endometriosis, risk increased three-fold (RR=3.12, 95% CI: 1.36-7.22) for women who were talc users and who had a history of endometriosis. However, this estimate is only based on a sample size of 22 cases and 9 controls and should be interpreted with caution. Contrary to the hypothesis that NSAIDS should have anti-inflammatory effects, OvCa risk increased with increasing frequency and duration of use

of NSAIDS, in line with other data available at that time.^{410,412,524} For example, it has also been found that human epithelial OvCa cells have very low expression of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2), which would reduce their sensitivity to the action of NSAIDS.⁵²⁴

Through in-person interviews with 812 women diagnosed with OvCa in western Washington state from 2002-2005 and 1,313 controls selected by random digit dialing, Rosenblatt et al.⁵²⁵ assessed the association between genital powder use and OvCa risk. Of note, this population does not overlap with that of Cook et al.,⁵¹² as the cases and controls in that study, which comprised three counties in western Washington, included women who were diagnosed with OvCa between 1986 and 1988. Measures of extent and timing of powder use were assessed, as was the relationship between powder use and the risk of disease subtypes according to histology and degree of malignancy. Perineal use of powder was associated with a slightly increased OvCa risk that was not statistically significant (OR=1.27, 95% CI: 0.97-1.66) (**Table 1**). Exposure on sanitary napkins or diaphragms was not associated with OvCa risk. Risk among perineal talc users was most evident among women with borderline tumors (OR=1.55, 95% CI: 1.02-2.37) (**Table 4**). No known pattern of risk was noted based on extent of use (assessed as years in which powder was used) or lifetime number of applications for invasive or borderline tumors or histologic subtypes (**Table 3**).

Kurta et al. (2012)⁵²⁶ used data from a large case-control study (known as the Hormones and Ovarian Cancer Prediction study) to determine whether fertility drug use impacts OvCa risk when accounting for parity, gravidity, cause of infertility and other factors, such as perineal talc use. Medical and reproductive histories were collected from 902 cases and 1,802 controls. Ever use of fertility drugs was not significantly associated with OvCa within the HOPE population overall (OR, 0.93, 95% CI, 0.65-1.35) or among the subset who reported seeking medical attention for infertility (OR, 0.87, 95% CI, 0.54-1.40). Perineal talc use was associated with OvCa risk in this population (OR, 1.40, 95% CI, 1.16, 1.69) (**Table 1**).

In addition to the study by Moorman et al.⁵²² which was published in 2009, several recent case-control studies^{40,521,527-529} have reported on talc-OvCa risk associations among racial/ethnic groups in addition to Non-Hispanic Whites, including Blacks and

Hispanic/Latinos. In a population-based case-control study conducted in Los Angeles county, Wu et al.⁴⁰ found that talc use was more common in Black women (41.3%) than in Non-Hispanic Whites (30.4%) or Hispanics (28.9%), consistent with data that reports the prevalence of genital hygiene product use to be highest among Black women.⁵³⁰ The authors⁴⁰ identified an elevated (adjusted OR=1.56 (95% CI: 0.80-3.04)), albeit not statistically significant, association with 1 or more years of talc use based on 128 Black cases and 143 Black controls.⁴⁰ On the other hand, OvCa risk was increased among Hispanic (adjusted OR=1.77 (95%CI: 1.20-2.62)) and Non-Hispanic White talc users (adjusted OR=1.41 (95% CI: 1.21-1.67)).

In 2016, Cramer and colleagues⁵³¹ evaluated the association between talc use and OvCa in a retrospective case-control study in Massachusetts and New Hampshire. A total of 2,041 cases with OvCa and 2,100 age- and residence-matched controls (identified through random digit dialing and driver license lists were included), though only 54% of controls enrolled. Interviewers asked subjects whether they “regularly” or “at least monthly” applied powder to the genital or rectal area, sanitary napkins or tampons, underwear, or areas other than the genital-rectal area. Interviewers were not blinded to case-control status, possibly contributing to bias. Additional details included type of powder, age begun, years used and applications per month. Lifetime exposure was estimated by multiplying frequency of applications per month by months used. This was divided by 360 (daily use coded as 30/month) to yield talc-years. Condom and diaphragm use as potential sources of talc exposure were also recorded.

Overall, genital talc use was associated with an OR of 1.33 (95% CI:(1.16- 1.52)) after adjustment for age, study center, and enrollment phase, particularly among serous and mucinous borderline tumors and invasive serous and endometrioid cancers.⁵³¹ A large increased risk for Black women was observed (unadjusted OR 5.08 (1.32-19.6)), based on an underpowered sample of 35 Black OvCa cases and 23 Black controls. The average age when women began use was 20 for cases and 19.8 for controls. Some women reported only using talc seasonally, but the questionnaire did not capture this. Of users, most reported body and genital use (27% of cases vs 23% of controls) rather than genital use only (5% of cases vs 4% of controls). Eighteen percent of cases (n=363 total women) and 15% of controls (n=316 women) reported use of Johnson’s Baby Powder or

Shower to Shower, with an adjusted OR=1.30 (1.10-1.54). Of note, for the substantial proportion of women who reported using “other brands” (including 14% of cases (n=279) and 11% of controls (n=233)), the OR was slightly higher 1.35 (95% CI: 1.12-1.64)⁵³¹ and the confidence interval wider due to the smaller sample size. Adjusted analyses initially only accounted for study matching factors (reference age, study center and enrollment phase), but later analyses showed that the parameter estimates did not change appreciably after adjustment for other potential confounders.

Association between genital talc use and EOC was *not* confined to any particular age or birth cohort, which is counterintuitive. The study also found that risk was greatest among pre-menopausal women (OR (95% CI)= 2.33 (1.32, 4.12)) and in post-menopausal women who used hormonal therapy (ORs (95% CI)= 2.57 (1.51, 4.36)). In attempting to explain the biologic credibility of the talc-OvCa association, Cramer et al.⁵³¹ pointed to data showing that inert particles the size of talc and sperm (known as microspheres) present in the vagina can migrate to the upper genital tract via a technique called hysterosalpingoscintigraphy.⁵³² Cramer et al.⁵³¹ then suggest that migration from the vagina is the “obvious” reason why talc can be found in diseased and normal ovaries. It is unclear why inanimate talc could/should be compared to microspheres, and by no means is this mechanism accepted universally. Indeed, IARC specifically noted that evidence to support this theory of migration was weak.⁴⁴⁹

Leveraging a population-based study known as the African American Cancer Epidemiology Study (AACES), Schildkraut et al.⁵²⁷ conducted the largest study to date to evaluate the association between body powder use (genital and non-genital) and OvCa risk among Black women in the US. The study included 584 cases and 745 controls and found that powder use was very common (62.8% of cases and 52.9% of controls). However, the study is limited in that a very small number of women reported having only used genital powder (43 cases and 44 controls). As such, the authors merged this exposure category with those who reported both non-genital and genital use of powder, creating an exposure category of “any” genital powder use. In the study, “any” genital powder use was associated with an increased risk of OvCa (OR = 1.44, 95% CI, 1.11-1.86) and serous histology (**Tables 1 and 4**). Additionally, a dose-response relationship was found for duration of use and number of lifetime applications (P< 0.05) (**Table 3**).

However, the significant positive association between “any” genital powder use and OvCa was only apparent among the cases and controls interviewed after 2014 (adjusted OR=2.91 (95% CI:1.70-4.97)), when the alleged talc-OvCa association was broadly discussed in the media. The authors posited that the press surrounding talc lawsuits could have sharpened memories of body powder use and increased reported use among cases. The proportion of cases reporting any genital powder use was increased substantially among those interviewed in 2014 or later (51.5% vs 36.5% prior to 2014), whereas use among controls was reportedly the same in both time periods (~34%). Schildkraut et al.⁵²⁷ also found stronger associations between “any” genital powder use and OvCa among post-menopausal women who reported hormone therapy (HT) use (OR= 2.68 (1.33, 5.40)) compared to non-users of HT (OR=1.24 (0.8, 1.79)).

In this study by Schildkraut and colleagues⁵²⁷, “only non-genital powder use” was associated with OvCa risk among nonserous OvCa cases (OR of 2.28 (95% CI, 1.39-3.74)); no dose-response relationship existed with frequency, duration or lifetime applications of “only non-genital powder use”. Schildkraut et al.⁵²⁷ suggested that non-genital powder use may be related to inhalation of inflammatory-associated exposures through the lungs. They went on to mention titanium dioxide (TiO₂), an inert particle that induces an inflammatory response upon inhalation and has been considered to be “possibly carcinogenic” to humans by IARC.⁴⁶⁸ Based on experimental evidence of enhanced inflammation due to exposure to TiO₂, increased asthma risk in mice,⁵³³ and epidemiologic data⁵²¹ supporting an association between powder use and asthma, it was postulated that the relationship between body powder use and respiratory conditions likely reflects an enhanced inflammatory response from powder usage. Of note, however, there is no clear evidence of an association between occupational exposure to TiO₂ and malignancies such as lung cancer.⁵³⁴ Schildkraut et al.⁵²⁷ ultimately suggested that the use of body powder may be an important modifiable risk factor for OvCa in Black women, but issues related to exposure misclassification and the small number of Black women exposed to “only” genital talc warrant investigation in larger populations of Black OvCa cases with more precise exposure assessment.

In a pooled analysis of 12 case-control studies, Peres et al.⁵²⁸ also examined the association between 17 reproductive, hormonal and lifestyle factors and OvCa risk by

race and ethnicity, using data from the African American Cancer Epidemiology Study analyzed by Schildkraut et al.⁵²⁷ and OCAC. It should be noted that controls were much younger than cases across racial/ethnic groups. Body powder exposure was classified as never use, any regular genital use, or only non-genital use, and only 8 of 12 studies had body powder exposure data. Black women were more likely to be obese, to report use of body powders and to have had a hysterectomy and a tubal ligation. Similar magnitudes of effects were observed among Black cases who had a hysterectomy (OR=1.64 (95% CI: 1.34, 2.02)) and Black cases who used powder in the genital area (OR=1.62 (95% CI: 1.32, 2.00)). Most Black women had high grade serous and not endometrioid OvCa. Of note, the finding in this paper of a positive association between hysterectomy and OvCa risk contradicts most epidemiologic studies, which have shown a protective effect for hysterectomy and other pelvic surgeries.^{35, 52, 77, 185, 259-263} A history of endometriosis was positively associated with OvCa risk in all racial/ethnic groups, with the largest OR in Blacks (OR=2.42, 95% CI:1.65, 3.55). The prevalence of benign gynecologic conditions that are indications for hysterectomy is likely to have confounded this association. Indeed, the incidence of uterine fibroids (also known as leiomyomas), a common indication for hysterectomy due to the painful symptoms, is 2-3 times higher among Blacks than Whites and contributes to a higher rate of hysterectomy in this population.^{535,536}

In a more recent publication, Peres et al.⁵²⁹ evaluated data from 4 case-control studies and 3 case-control studies nested within prospective cohorts in the Ovarian Cancer in Women of African Ancestry (OCWAA) Consortium to estimate race-specific associations and population attributable risks (PAR) for 10 exposures with OvCa risk. Ever/never use of body powder applied to genital areas was one of the exposures evaluated, though it was not ascertained in three studies (BWHS, MEC, and WHI). Moreover, participants diagnosed after 2013 were excluded to “circumvent reporting bias due to talcum powder and ovarian cancer lawsuits.” Although not statistically different, effect estimates for body powder use and PAR were higher among African American women (OR=1.36, 95% CI: 1.10-1.70; PAR=10.3%) than White women (OR=1.28, 95% CI: 1.15-1.43; PAR=6.5%). Stratifying by histotype and menopausal status revealed higher collective PARs among non-HGSC vs HGSC tumors and among pre-menopausal vs post-menopausal women. It is noteworthy that fibroids and douching were not

investigated as covariates despite recent reports,^{498,537} it is possible that adjustment for such exposures may have attenuated risk estimates.

In 2023, Leung and colleagues (which included Dr. Siemiatycki (an expert witness for the plaintiffs)),⁵³⁸ ascertained lifetime occupational histories for 491 OvCa cases and 897 controls as part of a population-based case-control study in Montreal, Canada. Linkage to the Canadian job exposure matrix (CANJEM) was used to assess participants' exposure to 29 agents in the workplace and OvCa risk. One of the agents evaluated was cosmetic talc. Only 15 cases and 16 controls were classified as ever being exposed to cosmetic talc, with a statistically insignificant association observed (OR=1.66, 95% CI: 0.80-3.46) on multivariable analysis. Furthermore, no statistically significant associations were detected between duration and cumulative exposure to cosmetic talc and OvCa risk. The authors⁵³⁸ concluded that "due to imprecision of our estimates and the presence of multiple correlated exposures, inferences of these results are limited" and suggest that "further population-based research is needed to evaluate possible hazards for female workers and occupations commonly held by women."

ii. Prospective cohort studies

The first prospective analysis of talc use and OvCa was published in 2000, by Gertig et al., based on the Nurses' Health Study (NHS),⁴⁹⁷ with the goal of addressing the limitations of recall bias in prior case-control studies by ascertaining talc exposure *prior* to case diagnosis. NHS is a prospective study of 121,700 female registered nurses in the US who were 30-55 years at enrollment in 1976. Talc use was ascertained in 1982 using a self-administered questionnaire: after exclusions, 78,630 women formed the analytic cohort and 307 women were diagnosed with OvCa during the follow-up period, which lasted until 1996.⁴⁹⁷ In 1982, 40.4% of the cohort reported ever use of talc and 14.5% reported ever using talc daily. No overall association was observed with ever use of talc and OvCa (multivariate RR=1.09, 95% CI: 0.86-1.37), and no increase in OvCa risk was observed with increasing frequency of use (Tables 1 and 3). Perineal talc use was shown to modestly increase the risk of invasive serous OvCa (RR 1.40 (95% CI)=1.02-1.91) (Table 4), but this risk was not statistically significant in covariate-adjusted analyses (RR=1.26 (95% CI=0.94-1.69)). Risk for OvCa was *not* increased among women who were perineal talc users but had never had a tubal ligation (RR=0.97, 95% CI: 0.71-1.32),

undermining the hypothesis that talc is transported to the ovaries via unobstructed fallopian tubes. The authors controlled for known and suspected OvCa risk factors in the analysis (parity, oral contraceptive use, tubal ligation history and body mass index). Limitations of this study include the fact that data were only available on the frequency of talc use per week at baseline and that a longer follow-up period is desired. Also, information was lacking about the age at which women began using talc and the duration of use, a limitation that is also present in most case-control studies.

In 2010, Gates and colleagues³¹ examined risk factors for OvCa risk by histologic subtype in the prospective Nurse's Health Study (NHS) I (1976-2006) and Nurses' Health Study (NHS) II (1989-2005). Women were 30-55 years old at enrollment in 1976 and were followed through June of 2006. With respect to talc use, the study only included the 108,446 NHSI participants who had been asked about talc use in 1982. Thus, the study essentially looked at the same cohort evaluated by Gertig et al., but followed them for 10 more years.⁴⁹⁷ No statistically significant associations were observed with genital talc use overall (RR=1.06, 95% CI:0.89, 1.28) or among any of the three main histologic subtypes, including invasive serous cancers (**Tables 1 and 4**). The latter result conflicts with findings from Gertig et al.,⁴⁹⁷ who found a modest association with invasive cancer. In other words, the elevated risk disappeared with 10 more years of follow-up.

The Gates et al.³¹ study has several strengths. First, the longer follow-up period means that the study would take account of any concerns about latency and that the women were older by the end of the study, putting them at greater risk of OvCa. Second, Gates used a different exposure definition of genital talc use (at least once a week)³¹ as opposed to "ever use,"⁴⁹⁷ which represents a more precise measurement. And third, gynecologic pathologists reviewed medical records to verify each OvCa diagnosis.

The authors' interpretation of their study raises a few concerns, however. Gates et al.³¹ suggest that the reason why their study did not find an elevated risk for serous invasive cancer with talc use may be due to a greater degree of exposure misclassification over the 24 years of follow-up since 1982, but that explanation is not logical or likely since the exposure (talc use) was defined at enrollment. In addition, it is unlikely that women over childbearing age would increase their talc habits. Indeed, data support a *decline* rather than an increase in talc use over time.^{497,539} Thus, the likelihood of exposure

misclassification whereby a non-user or user of <1 application/week became a user of ≥ 1 applications/week over the study period seems very unlikely. Similarly, although it is possible that a user of ≥ 1 talc application per week at the time of talc ascertainment (in 1982) could have become a user of a smaller number of applications or a non-user over the study duration, it is still important to consider the exposure history at baseline given the hypothesized latency of ovarian cancer. Finally, it is unclear why the authors commented that “the suggestive positive association with the mucinous subtype may reflect a longer latency period between talc exposure and development of mucinous tumors.” The association between talc and mucinous OvCa was *not* statistically significant (RR=1.50, 95% CI: 0.84, 2.66), and no biological rationale is provided to explain why there may be a longer latency period for certain histologic subtypes.

Houghton et al.⁴⁹⁹ assessed the association between self-reported perineal powder use (applied to genitals, sanitary napkins, or diaphragms, *and* duration of use) and OvCa risk prospectively in the Women’s Health Initiative Observational Study (WHI-OS) Cohort. This cohort comprises 61,576 post-menopausal women aged 50-79 at enrollment, which occurred between the years of 1993 and 1998. Women were followed until 2012 and therefore potentially had 14 to 19 years of follow-up (mean of 12.4 years). The outcome of interest (self-reported OvCa confirmed by physicians) occurred in 429 cases. More than half of the study population (52.6%) reported ever use of perineal powder; ever users were more likely to be heavier and more likely to have used oral contraceptives. No statistically significant OvCa risk associations were identified among ever users of perineal powder on the genitals (HR adj=1.12, 95% CI 0.92-1.36), sanitary napkins (HR adj=0.95, 95% CI: 0.76-1.20), or diaphragms (HR adj=0.92, 95% CI=0.68-1.23), compared with never users (**Table 1**). In a sensitivity analysis, the risk for invasive serous OvCa was not increased (HR_{adj}=1.13, 95% CI: 0.84-1.51) (**Table 4**), contrary to other findings.⁴⁹⁷ Furthermore, there were no associations with increased duration of use on genitals, sanitary napkins or diaphragms. The authors also conducted a sensitivity analysis restricted to women without tubal ligation; estimates were not increased, consistent with other studies.^{497,504} In summary, this large prospective study of the WHI-OS cohort⁴⁹⁹ found no association between perineal powder use and OvCa risk for any category of application, even with prolonged use.

The authors noted two primary limitations in the study: (1) frequency of talc use was not assessed; and (2) information about powder use was only collected at baseline. In assessing the second limitation, however, it is important to acknowledge that while it is possible that some never users could have begun using powder after baseline, that seems unlikely since women in this cohort were postmenopausal and would therefore be less likely to use powder on sanitary napkins or diaphragms. In an updated analysis of the WHI-OS cohort that aimed to develop and validate a hybrid risk classifier for identifying post-menopausal women at increased risk for invasive ovarian, fallopian tube, and peritoneal cancer, perineal talc use was dichotomized by duration (>10 years vs <10 years); talc use for >10 years was not associated with OvCa risk compared to talc use for <10 years (HR=0.97 (0.78,1.22)).⁵⁴⁰

The next individual prospective cohort study to evaluate the potential talc-OvCa risk association was the Sister Study, which enrolled 50,844 women who were between the ages of 35-74 at baseline in the US and Puerto Rico between 2003 and 2009, and reported having a sister diagnosed with breast cancer.⁴⁹⁸ Gonzalez et al. aimed to evaluate the association between douching and OvCa risk because douching has been reported to be associated with increased levels of urinary metabolites of endocrine-disrupting phthalates. At baseline, women were asked about douching and talc use during the previous year. Exposure information was nearly complete, with approximately 98% of participants having completed the personal care product portion of the questionnaire. During follow-up (median of 6.6 years), 154 women were diagnosed with OvCa, fallopian tube cancer or primary peritoneal cancer. Adjusted hazard ratios (HR) and 95% confidence intervals for OvCa risk were estimated. Douching was more common among talc users (HR=2.1, 95% CI: 2.0, 2.3), and douching at baseline was associated with increased subsequent risk of OvCa (HR=1.8, 95% CI:1.2, 2.8), whereas a reduced risk was observed between baseline perineal talc use and incident OvCa (HR: 0.73 CI, 95% CI: 0.44, 1.2). These findings suggest that talc use and douching appear to be positively correlated and support the possibility that earlier reports of positive associations between talc and OvCa risk were subject to confounding bias. The authors did note that null findings for a talc-OvCa association may be attributed to categorizing the exposure based on the 12 months prior to enrollment as a dichotomous ever/never variable, rather than a

quantitative measure of total applications. This study was also limited in that it may not have fully accounted for the long latency period of OvCa. Although douching or talc use habits at baseline may not reflect the entire period of risk, women who douched or used talc at baseline are likely to have been douching for a significant amount of time prior to enrollment. Detailed information about specific products used in douching was not collected and exposure to other chemical compounds (i.e., asbestos) was not estimated. This study has also been criticized for ascertaining limited details on pre-pubertal use of talcum powder, but the same could be said for most case-control studies on the topic that have been conducted to date. Finally, critics of this study have raised the issue that this cohort may not be representative of the general population because of its members' family history of breast cancer. But there is nothing about plaintiffs' hypothesis that would make it apply only to women at lower baseline risk.

In summary, douching was associated with increased OvCa risk in the Sister Study, but talc use was not. There is biological plausibility for an association between douching and OvCa risk. Douching has been associated with higher urinary levels of the endocrine disrupting chemicals (EDCs) known as phthalates among National Health and Nutrition Examination Survey participants.⁵³⁰ Furthermore, it has been suggested that douching could also move tissue, menstrual fluid, or foreign material up the reproductive tract, promoting infection. Of note, Gabriel et al.⁵⁴¹ used data from a case-control study in New England (2,040 cases and 2,100 controls) to estimate the association between self-reported douching, talc use, and the risk for OvCa and other adverse outcomes such as PID. The adjusted OR and 95% CI among women who douched but never used talc was not statistically significant (0.94 (0.76-1.16)) for OvCa overall or for specific histologic subtypes. However, the risk for OvCa overall and for serous invasive tumors was reported to be statistically significant among women who used talc but never douched, with OR (95% CI) of 1.28 (1.09-1.51) and 1.39 (1.14-1.69), respectively. When compared to women who were not regular douchers or talc users, ORs (95% CI) were 0.83 (0.52-1.33) for women who used talc and homemade douche and 1.53 (1.11-2.10) for women who used talc and store-bought douche. Cases who used talc and douched were five-fold more likely to have had PID than cases who were never users of either agent. Infectious agents that are possible contributors of PID such as *Chlamydia* were not reported on in

this study. Taken together, in this study,⁵⁴¹ douching was not found to be an independent risk factor for OvCa, but use of talc combined with store-bought douching products reportedly slightly increased OvCa risk. It is noteworthy that women in the study who douched were *more* likely to be parous and to have had a tubal ligation, factors associated with a *reduced* risk for OvCa; these factors were adjusted for. Also noteworthy are the small sample sizes for women who douched and the *lack* of a dose response between age at first use of talc or talc-years and OvCa risk among women who douched and women who did not douche. Collectively, there is not strong evidence for an association between talc or douching and OvCa risk in this study. The authors⁵⁴¹ discussed what they deem to be limitations of the Gonzalez study discussed above, including the fact that the Sister Study asked about genital exposure to talc *in the prior year*, even though 69% of cases and 56% of controls were post-menopausal and thus less likely to still be using talc, even if they had in the past.⁴⁹⁸ Gabriel et al.⁵⁴¹ also mention that only 12% of cases and 14% of non-cases in the Sister Cohort⁴⁹⁸ reported genital talc exposure, which is much lower than the other two prospective cohort studies that reported on the potential perineal talc use-OvCa association (40.4% in the Nurses' Health Study⁴⁹⁷ and 52.6% in the Women's Health Initiative⁵⁴²). It is possible that this is an underestimate because exposure was only ascertained for the year prior.

ii. Pertinent studies published since 2020 based on the Sister Study population.

A recent analysis on patterns of douching and genital talc use and reliability of self-reported exposure was conducted by O'Brien and colleagues⁵⁴³ using data from the Sister Study. At enrollment between 2003 and 2009, participants were asked to report use over the last year and during ages 10-13. In a follow-up questionnaire (2017-2019), participants were asked about use of douche and talc powder over their lifetime. Although women were fairly consistent in reporting use of these products at enrollment and around a decade or more later at the time of follow-up, approximately 10% and 13% of the women provided different answers about their reported use of douching and genital talc over time, respectively. Noteworthy was the fact that self-reported genital talc use increased from 27-28% to 32-33% among OvCa survivors, suggesting the possibility of recall bias. A main source of inconsistency also derived from 3,049 women (10% of the sample) who

initially reported using genital talc in the year before enrollment but subsequently responded that they did not use it during this period. As noted by the authors,⁵⁴³ limitations of this study included the narrow opportunity to assess reliability and possible lack of generalizability to other patient groups who are not highly educated or non-Hispanic White.

In 2024, Chang and colleagues⁵⁴⁴ published on associations between frequently used or “everyday” personal care products (PCPs) and incident hormone-sensitive cancers among women enrolled in the Sister Study. After an average of 11.6 years of follow-up, 277 incident OvCa cases, 4,226 breast cancer cases, and 403 uterine cancer cases were identified and included in the analysis. In the baseline questionnaire, information on self-reported use (over the prior 12 months) was ascertained for 41 PCPs including 12 beauty products, 7 hair products, 8 hygiene products (bath/shower gel, deodorant/antiperspirant, douche, mouthwash/rinse, shaving cream, talc (under arm), talc (genital), talc (other areas), and 14 skincare products. Responses for frequency of use included: “did not use”, “less than once a month”, “1–3 times per month”, “1–5 times per week”, and “more than 5 times per week.” Joint effects of PCP use on incident cancer development as well as the contribution of independent products on cancer incidence were estimated. A statistically insignificant association with OvCa incidence (HR=1.35, 95%CI=1.00, 1.83) was observed for the hygiene mixture, with douche as the main contributor (weight=57.6%). Across breast cancer, OvCa, and uterine cancers, associations with talc use were null, regardless of the mode of application (e.g., under arm, vaginal, or other) (Supplementary Table 4 of Chang et al.⁵⁴⁴) in adjusted models. Taken together, this study failed to support a statistically significant joint association with PCPs (or with individual products such as talc) and OvCa incidence.

More recently, O’Brien and colleagues⁶⁷² also applied quantitative bias analysis to data from the SIS study population to assess the influence of recall bias and misclassification on the association between intimate care products (e.g., douching and genital powder use) and OvCa risk under various scenarios based on the follow-up questionnaire described above. The most important finding of this study is the reaffirmance that prospective-looking data finds no association between talc use and ovarian cancer. For that data, the HR was 1.07 (0.84-1.35).

The authors did, however, find a statistically significant association of 1.82 (1.36-2.43) when using what they call “corrected” and “imputed” data. The premises of that analysis, though, are suspect. First, as explained above, many respondents offered contradictory responses regarding their talc use during the period surveyed at enrollment. For those respondents, the authors “corrected” the data by assuming that 80% of respondents who claimed no talc use at enrollment, but later in the follow-up questionnaire contradicted themselves actually did use talc. This assumption does not appear to be based on any data, however, and would fly in the face of what we know to be true of recall bias for women who were diagnosed with OvCa during the period between enrollment and follow-up.

Second, the authors attempted to plug the missing data that they had (resulting from over a quarter of the cohort not responding to the follow-up questionnaire) by conducting a “multiple imputation” analysis. I was unable to fully review and determine the accuracy of the multiple imputation analysis due to the scant details provided in the study and appendices. This imputation analysis itself may even be based upon data that are tainted by recall bias since it is trying to predict which women who claimed no use at enrollment would change their answer in the later follow-up, which would result in biased inputs in the predictive analysis. Given that, I agree with the authors’⁶⁷² statement in their conclusions that “there is still uncertainty as to how much recall bias and missing data could upwardly bias effect estimates.”

Third, the authors’ analysis does not and cannot identify a brand, agent, or mechanism that may drive the observed association. As the authors note, “[t]hose reporting talc use could be recalling products that contained talc, cornstarch, or a mixture, and women may have used different products at different times.”

I agree with a statement by O’Brien et al.⁶⁷² that “these results do not establish causality and do not implicate any specific cancer-inducing agent,” including talc.

iii. Summary regarding prospective cohort studies

Taken together, of four prospective cohort studies that published on the potential talc-OvCa association prior to 2020, none identified a positive, statistically significant association with OvCa^{31,497-499} (**Table 1**). Although prospective studies have numerous advantages, there are also limitations that warrant consideration in evaluating these

studies. First, there is greater potential for subject losses compared to other types of analytical studies; there is a need to make sure those lost are not systematically different from those in the original cohort in terms of exposure and outcome status. Second, there is also potential for exposure misclassification due to changes in exposure status during follow up; periodic reassessment of study exposures that can be modified by participants are therefore important in prospective cohort studies. Nonetheless, it is noteworthy that talc use is likely to be habitual, such that assessment at baseline is likely to be typical or characteristic of that individual and unlikely to change over time. In fact, Cramer et al.⁵²¹ reported year-round use to be the most common pattern (as opposed to seasonal or irregular use). And although these prospective studies may be criticized for not ascertaining the age at first use of talc, this information was only ascertained in a few case-control studies^{509,518,521}. Thus, even though the participants were only asked about their talcum powder use once, the data collected on perineal talcum powder application would have likely reflected chronic, habitual use.

Some of plaintiffs' experts^{545,546} have criticized the prospective cohort studies finding no association between talc use and ovarian cancer based on the belief that these studies involved an insufficient number of cases to detect a statistically significant result. These criticisms, which seem to focus on the low incidence rate of OvCa, are misplaced because they focus on rates for *all* women in the population, rather than rates for subsets of the population participating in these observational studies. The incidence of OvCa among women in prospective cohorts is actually higher than in the general population. More specifically, among 41,654 women in the SIS study,⁴⁹⁸ 61,576 women in WHI-OS,⁵⁴² and 108,870 women in NHSI,⁴⁹⁷ 429 cases occurred among 68,435 participants (0.62%) who reported exposure to talc, and 943 cases among 141,345 participants (0.66%) who reported no exposure to talc. Thus, 1,372 out of 212,100 women developed OvCa in these cohorts, which is far greater than the 10.9 per 100,000 women per year estimated from SEER.¹² Indeed, some of the cohort studies include women at higher risk for OvCa development due to age, family history of the disease or other risk factors. A higher incidence of OvCa in a study population actually suggests that the number of participants needed to detect a true effect (if one exists) is smaller. Indeed, this is the reason why epidemiologists design studies focused on higher-risk groups when studying rare

diseases. Taken together, well designed and executed cohort studies come closest to experimental studies in assessing cause-effect relationships, primarily because they are less prone to bias than other analytic studies.

D. Rationale for, and strengths and limitations of, pooled and meta-analyses.

In epidemiologic research, multiple observational studies that address the same research question can be integrated in a systematic way to provide an overall statistical summary of the results. The integration of individual studies can occur via a pooled analysis or a meta-analysis. In pooling, data are combined without being weighted as if data were derived from a single sample. Pooled analyses make use of the original data and can harmonize exposures and covariates across studies. In meta-analyses, data from subgroups or individual studies are weighted first, then combined; studies with more precise results (narrower confidence intervals) are given more weight.⁵⁴⁷ Such analyses are particularly useful when individual studies tend to be inconclusive due to small sample sizes or have inconsistent findings. Several published meta-analyses^{413-416,419,502,505,509,548-550} and pooled analyses^{500,504,551} of the talc-OvCa association have been conducted (**Table 2**) in order to estimate an overall summary estimate of effect and to assess heterogeneity.

Meta-analyses of observational studies should not necessarily be thought of as the “gold standard” method for reviewing a particular association. A key weakness of this method is that sources of bias are *not* controlled by the method: a meta-analysis *cannot* correct for poor design or bias in the original component studies. As has been discussed throughout this report, the main exposure of interest (use of talc in the genital area) is based on self-report. As such, the measurement of this exposure is imprecise, and the definition of the exposure varies between studies. Additionally, the strategy for adjustment of potential confounders was inconsistent across studies. Another potential limitation of meta-analyses is the reliance on published studies, which may exaggerate findings due to https://en.wikipedia.org/wiki/Publication_bias, since studies that observe negative or https://en.wikipedia.org/wiki/Statistically_insignificant results are less likely to be published than those showing a positive or statistically significant association. To evaluate the possibility of publication bias, funnel plots can be created; if publication bias does not

exist, it is expected that the plot will have a symmetric inverted funnel shape.⁵⁰¹ Finally, as Oleckno states, “meta-analyses may be influenced by the accuracy, thoroughness, objectivity, or knowledge of the researchers. They may also be limited by the quality or representativeness of the component studies. Biased studies or those with dissimilar subjects, interventions, or results may affect the accuracy of the findings from meta-analyses. Also, meta-analyses that exclude relevant studies may produce misleading findings. One potential problem in this regard is publication bias.”⁵⁰¹ Collectively, these caveats involved in conducting and interpreting meta-analyses can yield misleading information.

Heterogeneity in a meta-analysis refers to the degree of dissimilarity in the results of individual studies. In some situations, dissimilarities in results can be linked to inherent differences in the individual studies in terms of their population, design, definitions of the exposure and outcome, and/or analytic methods. In other situations, causes for the dissimilarities might not be easy to decipher. In any event, the more the level of heterogeneity increases, the more difficult it is to justify integrating the results. The classical measure of heterogeneity is Cochran’s “Q,” which is calculated as the weighted sum of squared differences between individual study effects and the pooled effect across studies, with the weights being those used in the pooling method. Unlike Cochran’s Q, another measure known as the I^2 statistic does not inherently depend upon the number of studies considered. I^2 is an intuitive and simple measure that quantifies the degree of inconsistency among results of studies comprising a meta-analysis.⁵⁰¹ The measure ranges from zero to 100%, with zero indicating no heterogeneity and 100% meaning maximum heterogeneity. If there is very little variation between studies, then I^2 will be low and a “fixed effects” model may be appropriate. A fixed effect model assumes that all the studies being examined are considered to have been conducted under similar conditions with similar subjects. Alternatively, a “random effects” model allows the study outcomes to vary in a normal distribution between studies. As such, many investigators consider the random effects approach to be a more natural choice than a fixed effects model. As will be discussed next, tests of heterogeneity were conducted for six meta-analyses^{413-416,505,550,552} and the pooled analyses,^{500,504} with three meta-analyses^{413,505,552} reporting

statistical significance for between-study heterogeneity (**Table 2**). Thus, study integration was not justified, and results of these meta-analyses should be interpreted with caution.

Findings from meta-analyses and pooled analyses. As alluded to in the prior section, because most of the previously described studies of talc and OvCa risk have had small sample sizes or have inconsistent findings, and because of media publicity that has enhanced interest in this issue, several meta-analyses^{413-416,502,505,509,548-550} and pooled analyses^{500,504} of the talc-OvCa association have been conducted to determine an overall summary estimate of effect and to assess heterogeneity (**Table 2**). In considering these studies, it cannot be emphasized enough that a meta-analysis or pooled analysis is only as rigorous as its component studies. A meta-analysis *cannot* correct for poor design or bias in the original component studies. Caution needs to be taken in the interpretation of the existing meta-analyses because the main exposure of interest (genital talc) is measured differently (and imprecisely) in the component studies and the strategy for adjustment of potential confounders is inconsistent across studies. The text that follows focuses on studies conducted since 2000 because they reported on tests of heterogeneity and estimated magnitudes of effect by study design.

A meta-analysis by Huncharek et al. in 2003⁴¹⁴ comprised 16 studies, including one prospective cohort and 15 case-control studies, and a total of 5,260 OvCa cases and 6,733 controls. The summary RR was 1.33 (95% CI: 1.16-1.45) for the association between perineal talc and OvCa risk (**Table 2**), and data showed a lack of a clear dose-response relationship. Sensitivity analyses showed no relationship between talc use and OvCa risk among hospital-based studies (RR=1.19, 95% CI: 0.88-1.41) and a modest association in population-based studies (RR=1.38, 95% CI: 1.25-1.52). Based on logic that the talc-OvCa hypothesis could be tested with better precision and validity if the exposure to the suspected carcinogen was directly applied/dusted on the reproductive tract as would be the case with a contraceptive diaphragm (as opposed to the perineal area), Huncharek and colleagues⁴¹⁵ subsequently focused on analyzing use of cosmetic talc on contraceptive diaphragms and the risk of OvCa in a meta-analysis of nine case-control studies consisting of 2,281 cases and 3,608 controls.^{136,337,506-509,512,517,553} None of the individual studies showed a statistically significant odds ratio (ORs ranged from 0.6 to 3.0), and the summary RR was 1.03 (95% CI: 0.80, 1.33), suggesting no association

between use of talc on diaphragms and OvCa risk (**Table 2**). Numerous sensitivity analyses were conducted (i.e., to eliminate one study¹³⁶ that did not explicitly provide data on talc use via contraceptive diaphragms or to exclude borderline OvCa cases from another study⁵¹⁷), but results remained insignificant. A methodological consideration is that the definition of control groups differed among the nine studies. Some reports defined controls as “never having used talc,”⁵¹⁷ while others used controls defined as never having used talc on diaphragms.⁵¹²

In 2008, Langseth and colleagues⁵⁰² published a meta-analysis of 21 studies comprising one cohort study and 20 case-control studies (14 population-based and 6 hospital-based) and reported an approximately 35% increase in risk of OvCa with genital exposure to talc. Similar to the prior meta-analysis by Huncharek et al.,⁴¹⁴ a statistically significant increased risk was only observed for the population-based case-control studies (OR=1.40 (95% CI:1.29-1.52)). However, because the test of heterogeneity was significant (P=0.036) and a fixed effect model was used for combining the results, these findings are not robust. The authors⁵⁰² ultimately concluded that the “current body of experimental and epidemiological evidence is insufficient to establish a causal association between perineal use of talc and ovarian cancer risk,” and suggested that “[e]xperimental research” be undertaken “to better characterize deposition, retention and clearance of talc.”

To determine whether OvCa risk increases with lifetime number of self-reported genital-powder applications for all histologic types of OvCa, Terry et al.⁵⁰⁴ conducted a pooled analysis that assessed 8 population-based case-control studies comprising 8,525 cases and 9,859 controls, with both groups having an average age of 55 years. Genital powder use was associated with a modest increased risk of OvCa overall (OR: 1.24 (95% CI: 1.15-1.33)) relative to women who never used powder (**Table 2**). Risk was elevated for invasive serous (OR:1.2 (95% CI: 1.09-1.32), endometrioid (OR:1.22, 95% CI:1.04-1.43) and clear cell tumors (OR:1.24, 95% CI:1.01-1.52), and for borderline serous tumors (OR:1.46, 95% CI: 1.24-1.72). Of note, the study by Terry et al.⁵⁰⁴ published in 2013 reports on 276 clear cell cases (which appear to include cases with invasive and borderline tumors) recruited from the New England Case Control (NECC) Study between 1992-2008. However, the study by Cramer et al.⁵²¹ published three years later, which also

reports on the NECC study (and three enrollment phases collectively spanning from 1992-2008) only had 114 invasive clear cell cases represented and did *not* report a significant association (OR=1.01, 95 CI: 0.65-1.57). Some possible reasons for the discrepancies in number include: (1) an error in the Terry paper;⁵⁰⁴; (2) different restrictions in these papers that are not readily apparent in the methods; or (3) a large proportion of clear cell cases being reclassified into other histologic groups. Cramer et al.⁵²¹ adjusted for endometriosis history, whereas Terry et al.⁵⁰⁴ did not. Terry et al.⁵⁰⁴ did perform tests for interaction, and did not observe significant interactions between genital powder use and endometriosis, parity, tubal ligation, hysterectomy, or menopausal status, with all P interactions greater than 0.1. (Other studies, such as one by Wu et al.,⁵²³ did not assess statistical interactions between endometriosis and talcum powder.) Among genital powder users, no significant trend ($p=0.17$) in risk was observed with increasing number of lifetime applications, and no increased risk was observed among women who only reported non-genital powder use. The authors concluded that genital powder use is a modifiable exposure associated with small to moderate increases in risk of most histologic subtypes of OvCa. The authors state that their findings are “consistent with and extend the findings of three meta-analyses^{414,502} that have reported an increased risk of epithelial ovarian cancer with genital powder use by including dose-response and histology-specific analyses.”

Three systematic reviews and meta-analyses were conducted to evaluate the talc-OvCa risk association and were published around the same time.^{413,416,505} **Table 2** summarizes and compares characteristics and findings of these three studies^{413,416,505} with one another and with the previous meta-analyses^{414,415,502} and pooled analysis.⁵⁰⁴ It is not surprising that the summary RRs are similar (ranging from 1.22 to 1.35) across most of these studies because they consider the same body of research.^{413,414,416,502,504,554} Of note, the aforementioned meta-analyses and pooled analysis^{418,435,448,414,502,504} focused on the association between self-reported perineal talc use and OvCa risk. Notably, their summary RRs differ from the meta-analysis by Huncharek et al. (2007),⁴¹⁵ which focused on use of talc on diaphragms and revealed an absence of an association (summary RR=1.03, 95% CI: 0.90, 1.33).

The systematic review by Berge⁴¹³ included 24 case-control studies and three cohort studies, totaling 15,019 women with OvCa. The summary RR for ever-use of

genital talc was 1.22 (95% CI: 1.13-1.30), but there was great heterogeneity identified ($P=0.017$, $I^2=40.1\%$) (**Table 3**). Among the case-control studies (six of which were hospital-based), the RR was 1.26 (95% CI: 1.17-1.35), while cohort studies failed to report an association (RR=1.02 (95% CI: 0.85-1.20)) (**Table 3**). Of note, the three cohort studies^{31,498,542} included in the meta-analysis covered 429 OvCa cases exposed to talc in the genital area and 943 unexposed OvCa cases, and it was well powered (99%) to detect a RR=1.25, had there been an effect to detect. Therefore, low power of cohort studies is not an appropriate explanation for heterogeneity of results, as the authors note. The only histological subtype that showed an association was serous carcinoma, with a RR of 1.24 (95% CI: 1.15-1.34), based on data from only 13 of the case-control studies (and no cohort studies).⁴¹³ No association was found between use of sanitary napkins or diaphragms and OvCa risk (RR=1.00, 95% CI: 0.84-1.16 and RR=0.75, 95% CI: 0.63-0.88, respectively). Moreover, no trend was identified with duration (RR=0.97, 95% CI: 0.82-1.12 for 9 studies) or frequency of talc use (RR=1.03, 95% CI: 0.82-1.25 for 5 studies).

In another recent systematic review and meta-analysis comprising 24 case-control (13,421 cases) and three cohort studies (890 cases, 181,860 person-years), Penninkilampi and Eslick⁴¹⁶ evaluated associations between any perineal talc use, long-term (>10 years) use, total lifetime applications, and use on diaphragms or sanitary napkins and OvCa risk. There was substantial overlap between individual studies included in the meta-analysis by Berge et al.⁴¹³ and the Penninkilampi and Eslick meta-analysis.⁴¹⁶ However, certain case-control studies^{417,506,509,522} and one cohort study by Gates et al.³¹ were included by Berge et al.,⁴¹³ but not Penninkilampi and Eslick,⁴¹⁶ while other case-control studies^{271,511,523,526} and a cohort study⁴⁹⁷ were only included in the Penninkilampi and Eslick⁴¹⁶ meta-analysis (**Table 5**). Any perineal talc use was associated with increased OvCa risk (OR = 1.31, 95% CI = 1.24, 1.39). Talc use on diaphragms or sanitary napkins was *not* individually associated with increased risk of OvCa. An association with ever use of talc was found in case-control studies (OR = 1.35, 95% CI = 1.27, 1.43), but not cohort studies (OR = 1.06, 95% CI = 0.90, 1.25) (**Table 2**). Dose-response data were available for 5 case-control studies; more than 3600 lifetime applications (OR = 1.42, 95% CI = 1.25, 1.61) were slightly more associated with OvCa

risk than <3600 (OR = 1.32, 95% CI = 1.15, 1.50). Among case-control studies, long-term talc use (>10 years) was associated with an increased OvCa risk, with an OR=1.25 (95% CI: 1.10-1.43), yet this is of a lower magnitude than any perineal use (OR=1.35). Pooling of two cohort studies^{497,542} showed an association between talc use and invasive serous OvCa (OR = 1.25, 95% CI = 1.01, 1.55); no other histologic subtypes had statistically significant findings in cohort studies. Of case-control studies that evaluated more than one histologic type, there were a few with statistically significant associations between talc and serous, endometrioid, or clear cell cancer, and none that identified associations with mucinous OvCa (**Table 4**).

The meta-analysis by Taher et al.⁵⁰⁵ was commissioned by Health Canada.⁵⁵⁵ It includes 27 original studies comprising three cohort studies and 24 case-control studies totaling 16,005 OvCa cases and 20,881 controls (**Table 2**). Studies that had analyzed overlapping study populations were assessed on a case-by-case basis for inclusion in the meta-analysis; the level of detail in the reported findings, including the sample size and publication date, were considered when deciding which study to include when overlap was identified. Taher et al.⁵⁰⁵ excluded several case-control^{136,140,170,417,511,556,557} and cohort³¹ studies that were included in one or both of the recently published meta-analyses^{413,416} (**Table 5**). Additionally, Taher et al.⁵⁰⁵ included case-control studies^{466,520} that had *not* been analyzed in other recent meta-analyses,^{413,416} including one study⁴⁶⁶ with poor quality (**Table 5**). The Taher et al. meta-analysis⁵⁰⁵ included an evaluation of the type of perineal use of talc powder (ever vs never use), the duration and frequency of talc use, tumor histology, tumor behavior, and the possible effect of menopausal status, hormone use, and pelvic surgery. Any perineal talc use was associated with increased OvCa risk (summary RR = 1.28, 95% CI = 1.20, 1.37), which is similar to the meta-analyses by Berge et al.⁴¹³ (summary RR=1.22) and Penninkilampi and Eslick⁴¹⁶ (summary RR=1.31). Similar to Berge et al., there was significant heterogeneity among studies ($P<0.0001$, $I^2=33\%$). A statistically significant association with ever use of talc was found in population-based case-control studies (RR = 1.34, 95% CI = 1.27, 1.41), but not in hospital-based case-control studies (RR=0.90, 95% CI: 0.78,1.17) or cohort studies (RR = 1.06, 95% CI = 0.90, 1.25), similar to prior meta-analyses^{413,416} (**Table 5**). Talc use on diaphragms or sanitary napkins was *not* associated with increased OvCa risk, with

RRs (95% CI) of 0.87 (0.72, 1.05) and 1.12 (0.91,1.39), respectively. No clear dose response was observed with increasing duration of talc use. Using data from studies that reported on associations with invasive serous histology, the RR was 1.32 (95% CI: 1.13, 1.54). In the final part of their manuscript, the authors⁵⁰⁵ applied the GRADE working group framework⁵⁵⁸ to the data in order to assess the quality of evidence derived from studies in the review. The GRADE assessment concluded there was very low certainty of evidence, meaning there is “very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.”⁵⁰⁵ The authors mention that observational studies are automatically scored lower than randomized clinical trials by GRADE⁵⁵⁸ and assert that their meta-analysis “lacks any serious issues with respect to inconsistency, indirectness, or imprecision” and continue to assert that “perineal exposure to talc powder is a possible cause of ovarian cancer in humans.” Again, it is worth reemphasizing that the *component studies* have serious issues with respect to inconsistency, indirectness and imprecision that cannot be overcome by combining them in a meta-analysis. Furthermore, the non-human studies mentioned by the authors (which are also discussed in this report) are not compelling.

Each of the three meta-analyses^{413,416,505} used the Newcastle-Ottawa Scale (NOS)⁵⁵⁹ to evaluate dimensions of study quality. The three dimensions evaluated in the NOS include *selection* (i.e., for case-control studies, is the case definition adequate, are the cases representative of the source population, is the selection and definition of controls adequate?; and for cohort studies, how representative is the exposed cohort of the community and how was exposure information ascertained?), *comparability* (of cases and controls on the basis of the design or analysis), and *exposure ascertainment* or *assessment of outcome* for case-control and cohort studies, respectively. For cohort studies, the NOS considers whether follow-up was long enough for outcomes to occur and the adequacy of follow-up in terms of possible bias introduced due to subjects lost to follow-up. The NOS assigns a maximum of four points for selection, two points for comparison, and three points for exposure or outcome, for nine points total despite commentary about a maximum score of 10 by Penninkilampi and Eslick.⁴¹⁶ Studies with at least seven points were considered high quality by Taher et al.⁵⁰⁵ Taher et al.⁵⁰⁵

estimated that studies of talc-OvCa risk that had an NOS ≥ 7 had a higher RR (1.32 (95% CI: 1.25, 1.40)) than studies with an NOS < 7 (RR=1.21 (95% CI: 1.05, 1.39)).

Of note, there are 35 individual studies that are part of at least one meta-analysis that used the NOS to evaluate study quality (**Table 5**). Only 15 of these studies (42.8%) had an average NOS ≥ 7 . Of the 35 individual studies, 16 identified a positive weak or moderate association between talc and OvCa risk, but only 9 of these studies had an NOS ≥ 7 . Of the remaining 19 studies that reported an absence of an association, 6 had an average NOS ≥ 7 , including the two prospective cohort studies by Gates³¹ and Houghton,⁵⁴² which have the longest follow-up time of the four cohort studies.^{31,497-499} Taken together, it is concerning that the majority (57.2%) of heavily cited epidemiologic studies of the talc-OvCa association represented in **Table 5** rated poorly on the NOS scale; yet, the scientific community is being asked to rely on these data to assess causality.

In sum, the increased risk observed in these meta-analyses is restricted to case-control studies and is likely due to bias and/or confounding. It must be reiterated that meta-analyses are only as valid as their component studies and that combining studies that use different and imprecise methods to measure the exposure reduces the reliability of a meta-analysis.

Finally, O'Brien and colleagues⁵⁰⁰ recently performed the largest investigation of the talc-OvCa relationship by conducting a pooled analysis of four large prospective cohort studies (NHSI, NHSII, WHI-OS and the Sister Study, SIS) containing 252,745 women, of whom 2,168 developed OvCa during the study periods. The included study periods for NHSI, NHSII, WHI-OS, and SIS were 1982-2016, 2013-2017, 1993-2017, and 2003-2017, respectively. This extended available data for each study by 20 years for NHSI, 5 years for WHI-OS and 3 years for SIS. This study, published in the leading journal JAMA, found a decrease in perineal talc use over time, with cohorts that enrolled the oldest participants (WHI-OS; median age of 63 at assessment of talc use) having the highest proportion of perineal powder users (53%) compared to lower percentages of use in cohorts that enrolled participants at younger ages (NHSII, 26% (median age: 58) and SIS, 27% (median age: 55)). The exposure (talc use) was measured differently in each study; NHSII, SIS and WHI-OS asked women about duration of use, and NHS, NHSII and

SIS asked women about frequency of use. When comparing OvCa incidence in perineal talc users versus non-users, a non-statistically significant HR of 1.08 (95% CI:0.99-1.17) was detected. No evidence of dose response was observed when examining duration and frequency of talc use. When the analysis was restricted to women with patent reproductive tracts (i.e., those who had their uterus and fallopian tubes intact and therefore were reported to have an unobstructed physical pathway between the genital areas and ovaries), the HR among ever users (compared to non-users) was 1.13 (95% CI:1.01-1.26). Among women without patent reproductive tracts, the HR for ever users was 0.99 (95% CI: 0.86-1.15) compared to non-users, and P-heterogeneity comparing results for women with patency versus without patency was 0.15. This confirms that no statistically significant association exists between use of powder in the genital area and OvCa risk. Furthermore, in stratified analyses for the association between long-term use (≥ 20 years) of powder in the genital area (with never users as the referent group), HRs were not statistically significant among patent users (HR=1.00, 95% CI: 0.76, 1.32) or non-patent long-term users (HR=1.03, 95% CI: 0.75-1.41). In an editorial by Gossett and del Carmen that examines the evidence from the O'Brien pooled analysis,^{500,560} the authors astutely point out that "it is not possible to equate a patent reproductive tract with exposure and a nonpatent reproductive tract with nonexposure. Women who undergo tubal ligation or hysterectomy (nonpatent) and use powders in the genital area cannot be assumed to have started using them only after their surgeries . . . this is highly unlikely as women often begin use of powder in the genital area in adolescence." Gossett and del Carmen also pointed out that the "subgroup analysis suggesting that women with intact reproductive tracts who used powder in the perineal area developed ovarian cancer more frequently than non-users is below the effect size that epidemiologists generally consider important and should not be selectively highlighted by the statistically unsophisticated reader as evidence of a relationship."⁴⁸⁵ (Furthermore, it is noteworthy that when the outcome was confined to medically confirmed cases, the association was attenuated and no longer statistically significant (HR:1.05 (95% CI: 0.96-1.16) for ever versus never use). Additionally, notable differences in estimates were *not* found when evaluating invasive status, tumor location or histology or when restricting analyses to women with patent reproductive tracts among medically confirmed cases. Taken together, this large, well-

powered, and rigorously conducted study with significant follow-up time does *not* support a causal association between perineal talc use and OvCa risk.

Of note, prior pooled and meta-analyses^{504,505,551} also commented on associations between powder use in the genital area and OvCa risk in women with and without patent reproductive tracts. After excluding those who first started using genital powder following hysterectomy or tubal ligation, Terry et al.⁵⁰⁴ found that results were similar to the overall analysis (OR = 1.36, 95% CI: 1.18–1.57 for the 4th vs 1st quartile of cumulative number of lifetime talc applications compared to the original overall estimate OR = 1.32, 95% CI: 1.16–1.52). Taher et al.⁵⁰⁵ had reported an inverse association between talc and OvCa among women with a tubal ligation (OR = 0.64, 95% CI: 0.45–0.92), and when they investigated studies that reported estimates from women with a history of either hysterectomy or tubal ligation, the estimate from the meta-analysis approached null (OR = 1.06, 95% CI: 0.78–1.42). Similar estimates were reported by Davis et al.⁵⁵¹ when analyses were restricted to women with patent reproductive tracts (OR = 1.27, 95% CI: 1.09–1.48) versus those with a history of tubal ligation or hysterectomy (OR = 1.42, 95% CI: 1.17–1.72; *P* heterogeneity=0.31). Taken together, due to the challenges with establishing a clear sequence of events in use of powder in the genital area relative to hysterectomy or tubal ligation, especially in the case-control studies, interpretation of these findings is difficult. Furthermore, such analyses do not consider alternate explanations for the risk reduction afforded by tubal ligation, such as induction of quiescence in the epithelia of the fallopian tube fimbria.²⁸⁹

Strengths of the pooled analysis by O'Brien et al.⁵⁰⁰ include its large sample size (2,168 OvCa cases that occurred over 3.8 million person-years) and lengthy follow-up time, which is much longer than a prior meta-analysis of published NHSI, SIS, and WHI-OS results (890 cases over 182,000 person-years).⁴¹⁶ Study limitations include the variation in the measurement of the exposure, specifically the duration, frequency and type of powder use. Despite these differences, there was no evidence of between-study heterogeneity. Another possible limitation of this analysis is that talc use was only measured at baseline and not over time (i.e., none of these studies collected data on talc use at timepoints after baseline/enrollment). As noted earlier, however, most women begin talc use by the age of 20, diminishing concerns about differential miscalculation.

Moreover, participants from these prospective studies have been reported to accurately self-report data on exposure history, increasing the internal validity of findings.⁵⁰⁰ Other supposed limitations are spelled out in letters to the editor,^{561, 562} some of which were submitted by experts or consultants for plaintiffs in talc litigation, and many of which would apply even more strongly to case-control studies. In summary, this large-scale, robust and comprehensive analysis of pooled data from women in four US cohorts with ample follow-up time for most study participants did *not* reveal a statistically significant association between self-reported talc use and incident OvCa.

In 2021, Davis and colleagues⁵⁵¹ published results from a pooled analysis of four population-based case-control studies and one prospective cohort study participating in the Ovarian Cancer in Women of African Ancestry Consortium (OCWAA). A positive but non-statistically significant association was observed between powder use in the genital area and OvCa in African American women (OR=1.22, 95% CI: 0.96-1.55). Among White women, the OR was 1.34 (95% CI: 1.16-1.56), with a combined estimate of OR=1.31 (95% CI: 1.15-1.38) overall. No clear dose response was observed. Consistent with previous reports, African American women were more likely to report ever use of genital powder (34% of African American non-cases versus 31% of White non-cases), but effect estimates were similar between the two racial groups (OR = 1.22, 95% CI: 0.97–1.53 in African American women and OR = 1.37, 95% CI: 1.1–1.57 in White women). In analyses restricted to HGSOE, Davis et al. reported increased associations for both African American (OR = 1.30, 95% CI: 1.00–1.68) and White (OR = 1.32, 95% CI: 1.13–1.56) women. Non-serous tumors were positively associated with powder use in White women (OR = 1.38, 95% CI: 1.15–1.66), but not African American women (OR = 1.08, 95% CI: 0.78–1.51).

In 2022, another systematic review and meta-analysis was published by Drs. Woolen, Lazar, and Smith-Bindman⁵⁵⁰. Noteworthy is the fact that the senior author, Dr. Smith-Bindman, serves as a paid expert witness for the plaintiffs in the talcum powder litigation and included a prior version of this meta-analysis⁵⁵⁰ as part of her litigation report in the multidistrict litigation before the article was subsequently published. The first author, Dr. Woolen, was a radiology fellow when preparing the meta-analysis and reported no substantive expertise in the areas of epidemiology, talcum powder, or OvCa prior to

conducting this review. In the methods section of their meta-analysis, the authors⁵⁵⁰ stated that the analysis was focused on estimating the association between “frequent” perineal talcum powder use and OvCa risk. The authors defined frequent use as “[equal to or greater than] 2 times per week,” but they did not explain why this measure of frequency was chosen over other measurements, including at least 1 time per week, which may have allowed for the inclusion of more data (including data from the cohort studies). Moreover, the specific duration (e.g., over the lifespan or other time period) for this measure of frequency was not stated at the outset of the investigation.

Data from eleven studies reportedly met the authors’⁵⁵⁰ inclusion criteria, including 10 retrospective case-control studies (Booth, 1989; Chang, 1997; Cook, 1997; Cramer, 2016; Harlow, 1992; Mills, 2004; Rosenblatt, 2011; Schildkraut, 2016; Whittemore, 1988; Wu, 2009). Woolen et al.⁵⁵⁰ stated that none of the “four prospective cohort studies as reported in O’Brien⁵⁰⁰ met their “pre-specified” criteria of perineal use > 2 times/week. Thus, the authors stated that they contacted Dr. O’Brien and requested primary data from these studies for women with the “highest frequency exposure group” of perineal talcum powder⁵⁵⁰. See article p. 2 (“Data were included from the highest reported talc use category to obtain as close to daily use as possible and the referent group were women who reported no talc exposure.”). In a return email from Dr. O’Brien to Drs. Woolen and Smith Bindman dated 4/9/2020, Dr. O’Brien indicated that “there were only 2 “exposed” (used powder ≥ 5 times/week) cases from the SIS study” (i.e., the highest exposure category requested by Woolen and Smith Bindman), and therefore the SIS study would not “contribute much information”. Dr. O’Brien also informed Drs. Woolen and Smith-Bindman that NHS2 would not be able to contribute to this analysis because NHS2 asked about weekly usage, which did not meet the authors’ criteria. As mentioned previously in this report, the study--specific estimate for frequent versus never use from the NHS2 cohort was statistically insignificant (0.81 (95% CI: 0.47-1.38)).⁵⁰⁰ Dr. O’Brien also reiterated that “WHI did not collect data on frequency of use.” Thus, Dr. O’Brien explained that the only cohort data that met the authors’ email request was from NHSI, because the study participants were allowed to indicate that they used powder daily.

Woolen and Smith Bindman⁵⁵⁰ proceeded to use the data generated with the assistance of Dr. O’Brien from this very selective subset of NHS I participants, which

again was all that could be used from the cohort studies given the poorly explained parameters of the study design (defined in methods as greater than or equal to two times per week, and further elucidated in the study as the “highest frequency exposure group” and “as close to daily use as possible”). Then, in the Woolen paper itself, the authors further selectively analyzed the unpublished data provided by O’Brien by including only the data “on women with intact fallopian tubes” (i.e., patent reproductive tracts) from this highest exposure group (referred to in the Woolen study as “O’Brien NHSI”). In the Woolen study, the authors claimed that their decision to further parse the data to women with intact fallopian tubes was done in order “to harmonize with other publications,” but they did not clearly explain how this harmonized the data with the other studies (which were not similarly restricted). As to the other case-control studies used in the Woolen study, they used the highest exposure level, which ranged from 4-7 times/week to daily. See Table 2 & comments below Figure 2. Their usage of the highest exposure category from each study is different from their⁵⁵⁰ pre-specified criteria of perineal use >2 times/week, and they never clearly explained the basis for this divergence from their methods. Ultimately, the Woolen study reported that “frequent” perineal use of talcum powder (as they defined that term) is associated with a pooled adjusted OR of 1.47 (95% CI 1.31, 1.65), and they erroneously concluded that “the results support women avoiding frequent use of talcum powder in the perineal area.”

The study by Woolen et al.⁵⁵⁰ is methodologically flawed in its design and interpretation for numerous reasons, a sentiment commented on by other scientists⁵⁶³ and peer reviewers for several journals including *JAMA Internal Medicine*⁵⁶⁴ and *Annals of Internal Medicine*⁵⁶⁵. I strongly agree with the peer reviewers⁵⁶⁴ that it was inappropriate “to conclude causality solely on the basis of a pooled (and biased) estimate of relative risk.” I agree that the Woolen article suffers from “significant methodological concerns.” First, there is bias in the studies and participants included in the analysis. As noted above, one of the biggest concerns relates to the selection of data from the prospective cohort studies. For example, since NHS I data⁴⁹⁷ categorized genital powder use based on three frequency levels (<once per week, 1-6 times/week, or daily use) versus never use, it is concerning that Woolen et al.⁵⁵⁰ was designed to only permit the inclusion of data from daily users, and excluded participants who reported use at other frequencies, including

those who reported using talc 1-6 times per week. This design attempts to bias results away from null to support their hypothesis. The NCI PDQ⁵⁶⁶ appears to share my concern noting that Woolen used a “highly selected subset analysis of one prospective study that was inconsistent with the main findings of the original [O’Brien] report,” and that “because of the structure of this analysis⁵⁵⁰, the “results should be interpreted with care.”

Additionally, peer reviewers⁵⁶⁴ of the Woolen article noted that because the “[r]esults on frequent use of talc are reported in the pooled analysis of cohort studies by O’Brien et al., “[t]he use of an unpublished result from a subgroup analysis is not justified.” Additionally, as mentioned previously, results from the Sister Study⁴⁹⁸ were not included because only 2 women were in the *highest* exposure category, which the Sister Study defined as “more than 5 times per week.” Given Woolen’s definition of “frequent” and the authors’ further restriction and focus on only the highest exposure categories, users who reported 1-5 times per week were excluded. It is worth reiterating that Gertig et al.⁴⁹⁷ did not detect a statistically significant association between the highest frequency group (daily users) and OvCa risk (multivariate RR (95% CI): 1.12 (0.82-1.55)), and no trend was observed with increased frequency of use. Further, when O’Brien et al.⁵⁰⁰ conducted their pooled analysis of 1,519 cases and 179,712 non-cases who used powder ≥ 1 x/week, the adjusted OR was non-significant (HR:1.09, 95% CI: 0.97-1.23). It was only when they⁵⁰⁰ restricted to cases with patent reproductive tracts and powder use ≥ 1 x/week that a significant association was detected (HR 1.19, 95% CI:1.03-1.37). That association was no longer significant when restricted to all medically confirmed cases or any histology or subgroups. Additionally, from the outset of their meta-analysis, Woolen et al.⁵⁵⁰ did not indicate that they would systematically be focusing on effect estimates from women with patent reproductive tracts who were daily users, but selectively chose to do so for the unpublished O’Brien data by including only the adjusted OR for patent women (1.40 (95% CI: 1.17,1.68)) in the meta-analysis rather than the adjusted OR for all women (1.27, 95% CI: 1.09-1.49) as shown in Figure 2 by Woolen et al.⁵⁵⁰

Woolen ⁵⁵⁰ concludes that the “magnitude of the higher association in our study compared to prior case-control and cohort meta-analyses was likely due to our focus on frequent rather than any talcum powder users, inclusion of quality studies, and consistent definition of the exposure.” But, their definition of exposure was far from consistent and

differed from the exposure level they set out to investigate (e.g., $\geq 2x/week$). Woolen et al.⁵⁵⁰ report on Newcastle Ottawa Scale (NOS) scores they calculated for each study; their scores tend to be biased, with higher scores reported for studies that were included compared to NOS scores calculated in prior meta-analyses (**Table 5**). In sum, the meta-analysis by Woolen et al.⁵⁵⁰ is methodologically flawed in its design and appears to reflect the authors' bias.

Several reviews^{567,568} and commentaries⁵⁶⁹⁻⁵⁷¹ on the purported talc-OvCa association have also been published since 2020, but will not be discussed in detail because no new primary data was added. Briefly, Tanha et al.⁵⁶⁷ conducted an umbrella review of two systematic reviews and reported that perineal talc use was associated with an increased risk for OvCa (OR 1.29, 95% CI 1.24-1.35, $P < 0.001$). Lynch et al.⁵⁶⁸ also conducted a systematic review of the potential carcinogenicity of genitally applied talc in humans by evaluating and integrating epidemiological, animal, and mechanistic literature on talc and female cancers. The authors concluded, "Integrating all streams of evidence . . . yielded classifications of suggestive evidence of no association between genital talc application and risk of OvCa cancer at human-relevant exposure levels. They also found "insufficient evidence to determine whether a causal association exists between genital talc application and cervical cancer based on a smaller but largely null body of literature." The review by Lynch et al.⁵⁶⁸ is criticized by Plaintiff experts^{572,573} for not adding new primary data and not conducting another meta-analysis of the same component studies. Dr. Siemiatycki⁵⁷² also criticized the commentary by Goodman et al.⁵⁷⁴ for similar reasons. It is not surprising that undue criticism is being raised against commentaries^{569,574} and reviews⁵⁶⁸ whose conclusions regarding causality do not align with conclusions of the Plaintiff's experts. I do not believe that another meta-analysis of the same body of epidemiological data would yield additional helpful information on this topic.

Summary of histology-specific estimates for powder use in the genital area from pooled and meta-analyses. As mentioned throughout this report, OvCa encompass several histotypes, which have different cells of origin and risk factors. Most studies published since 2000 have included histology-specific estimates, with serous OvCa being the most common. In recent meta-analyses, Penninkilampi and Eslick⁴¹⁶ reported that ever use of talc was positively associated with serous carcinomas (OR =

1.32, 95% 1.22–1.43) and endometrioid tumors (OR = 1.35, 95% CI: 1.14–1.60), but a statistically significant association was not observed for mucinous (OR = 1.12, 95% CI: 0.94–1.33) or clear cell (OR = 1.02, 95% CI: 0.75–1.39) carcinomas. Similar findings were observed by Berge et al.⁴¹³, including a positive association between talc use and serous carcinoma (RR: 1.24, 95% CI: 1.15–1.34), but no statistically significant associations with endometrioid carcinoma (RR: 1.15, 95% CI: 0.91–1.39), mucinous (RR = 0.96, 95% CI 0.73–1.18) or clear cell cancer (RR = 0.98, 95% CI: 0.72–1.23). A positive association with serous tumors was reported in the Taher et al.⁵⁰⁵ meta-analysis (OR = 1.35, 95% CI: 1.21–1.50). Taher et al.⁵⁰⁵ also observed a non-statistically significant association for mucinous tumors (OR = 1.17, 95% CI: 0.82–1.67). In the Terry et al.⁵⁰⁴ pooled analysis, ever use of powder in the genital area was associated with serous (OR = 1.20, 95% CI: 1.09–1.32), endometrioid (OR = 1.22, 95% CI: 1.04–1.43) and clear cell (OR = 1.24, 95% CI: 1.01–1.52) carcinomas, but not mucinous (OR = 1.09, 95% CI: 0.84–1.42). In the pooled analysis by O'Brien et al.,⁵⁰⁰ which included updated data from the prospective cohorts, a non-statistically significant association was observed between ever use of powder in the genital area and medically confirmed serous ovarian cancers (HR = 1.10, 95% CI: 0.97–1.25), endometrioid cancers (HR = 1.15, 95% CI: 0.83–1.58), clear cell carcinomas (HR = 1.17, 95% CI: 0.73–1.89) and mucinous tumors (HR = 1.03, 95% CI: 0.69–1.54). When O'Brien and colleagues' histology-specific analyses were limited to women with patent reproductive tracts,⁵⁰⁰ the HRs did not substantially change and remained statistically insignificant. The Davis et al.⁵⁵¹ pooled analyses reported an increased risk for serous tumors in African American (OR = 1.30, 95% CI: 1.00–1.68) and White women (OR = 1.32, 95% CI: 1.13–1.56), but did not separately evaluate the other histotypes. Collectively, these results suggest a weak, inconsistent positive association between talc use and serous ovarian cancers and potentially endometrioid tumors. The relationship between talc use and the rarer mucinous or clear cell tumor histotypes is even more attenuated. The finding of a weak positive association with clear cell observed by Terry et al.⁵⁰⁴ is certainly not in line with the bulk of data in this area.

E. Lab Based *In Vivo*, *In Vitro* & Human Studies of Talc, Asbestos and OvCa.

The importance of integrating data from multiple disciplines. Advances in molecular biology, genetics and toxicology have allowed the scientific community to better understand the complexity behind human disease initiation and progression and more effectively open the “black box” of mechanisms behind exposure-disease associations. In fact, nowadays, molecular experimentation can reinforce epidemiologic findings by providing supportive evidence for a mechanistic hypothesis, lessening the need for repetition among numerous observational studies. Consistent with recommendations for how researchers should apply causation analysis in the 21st century,⁵⁷⁵ this report integrates data, knowledge and reasoning from multiple disciplines and approaches (including epidemiology, molecular biology, genetics, toxicology and statistics) to assess a possible causal association between talc and OvCa. To meaningfully address other fields of scientific inquiry, I have also reviewed animal, laboratory and toxicology studies on talc, asbestos and OvCa. Such *in vivo* and *in vitro* studies were performed to assess the biological impact of talc on tissue and cells removed from animals and humans to ultimately determine whether talc can cause cancer. Collectively, studies from other disciplines do *not* support plaintiffs’ claims that talc caused their OvCa diagnosis.

In addition to studying causality in humans with and without an exposure and outcome of interest, laboratory studies can be performed to evaluate *in vivo* whether animals exposed to a substance (often in large doses) develop tumors or other conditions. Investigators can also conduct *in vitro* studies to expose normal cells to the substance to see if it causes the types of changes that are seen in cancer cells. Genotoxicity describes the property by which chemical agents damage the genetic information within a cell, causing mutations. *In vitro* toxicology studies that suggest a mode of action such as genotoxicity or altered gene expression can support an association found in an epidemiologic study.⁵⁷⁵ However, numerous lines of data from animal, toxicology and human laboratory studies to be described next provide data *in opposition to and/or refute* the talc-OvCa hypothesis.^{491,542,576-579 576,580-583}

Animal Studies. The limited studies that exposed lab animals (rats, mice and hamsters) to talc have not supported the theory that talc is a carcinogenic agent. For

example, Hamilton⁵⁸² injected saline containing talc (100 mg/ml) into the bursa, a membranous sac of peritoneum in the ovarian cavity of rats. In a subset of treated animals (but no controls), small areas of papillary cystic changes were noted in the surface epithelium of the ovary. Histological assessment revealed that the cysts were not derived from the ovary; rather, they arose due to distension of the bursal sac caused by an accumulation of follicular fluid. Polarized light and electron microscope analysis confirmed the presence of talc in the surface epithelium, ovarian cortex and connective tissue matrix in the bursa. It was suggested that the cystic changes may be related to constant exposure to steroid hormones that accumulated in the intrabursal space. Importantly, the authors did not observe evidence of cancer. Considering the relatively high amount of talc that was directly applied to the bursal sac, it is unclear how representative this study is to humans. In another study, male (n=50) and female (n=50) Syrian golden hamsters were exposed to cosmetic-grade talc aerosol (with a mean concentration of respirable aerosol fraction of 8 µg/liter) for 3, 30, or 150 min/day, 5 days/week for 30 days, or for 30 or 150 min/day until they died or for a maximum of 300 days.⁵⁸⁴ Exposure to talc aerosol did not appear to have an impact on body weight or survival in talc-exposed groups versus sham-exposed controls (which were exposed to filtered room air instead of talc aerosol). Furthermore, only a few neoplasms were detected that varied by type among the treatment groups and controls. No ovarian tumors were detected. Hamsters were reported to have received cumulative exposures ranging from 15-6000 mg/hr/m³, which exceeds the average of weekly infant exposures by 30-1,700 times. Additionally, in a later study by Wehner et al. (1980),⁵⁸⁵ there were no histopathologic changes in the lung, heart, renal tissue or uterus of hamsters exposed to aerosols containing 8 mg/m³ of cosmetic-grade talc for 150 minutes per day, 5 days per week, for 300 days. Collectively, the fact that significant doses of talc failed to show any evidence of pro-oncogenic effects is strong evidence against plaintiffs' experts' arguments.

Endo-Capron and colleagues⁵⁸⁶ reported on experiments that were conducted to evaluate the carcinogenic potency and genotoxicity of talc samples. Inoculation of talc into the pleural cavity of rats was performed, and genotoxicity was evaluated by assessing chromosomal effects in cultures of rat pleural mesothelial cells (RPMC) using sister chromatid exchange (SCE) analysis. The experiments included a total of 152 Sprague-

Dawley rats that were divided into the following exposure groups: 35 received intrapleural injection of 20 mg talc; 40 received intrapleural injection of saline; 38 received no injection; and 39 received Canadian chrysotile. No pleural tumors were identified in the talc-treated group, whereas approximately 25% of rats exposed to chrysotile fibers developed mesothelioma. Control groups did not develop tumors. A statistically significant enhancement of SCE was *not* observed in cultures treated with talc, even at 15 $\mu\text{g}/\text{cm}^2$, which was the highest concentration. However, SCE enhancement was observed with mitomycin C, a known mutagenic agent. Thus, in the present experiment, talc did *not* show tumorigenic potency or genotoxicity.

In 1993, the National Toxicology Program (NTP) started publishing inhalation studies they had designed and conducted to evaluate the toxicologic potential and carcinogenic activity of talc (non-asbestiform, cosmetic-grade) in F344/N rats and B6C3F mice.^{581,587} F344/N rats were exposed to aerosols 6 hours/day, 5 days/week for up to 113 weeks (males) or up to 122 weeks (females). B6C3F mice were exposed in a similar fashion for up to 104 weeks. Exposure levels for each group were 0, 6 or 18 mg/m^3 . Outcomes included body weight, survival rate, and non-neoplastic and neoplastic effects examined pathologically. Mean body weights of male and female rats from the 18 mg/m^3 group were slightly lower than controls after week 65. Body weights of exposed groups of mice were similar to controls. Survival of male and female rats and mice was similar to that of controls. Granulomatous inflammation, interstitial fibrosis, and alveolar epithelial hyperplasia were non-neoplastic effects observed in the lungs of male and female rats who had inhaled talc, while chronic inflammation and macrophage hyperplasia were observed in male and female mice exposed to talc. Neoplastic effects were reported in rats, but not in mice. It was speculated that the lung talc burden of rats was greater than that of mice due to anatomical and physiological differences.⁵⁸⁷ Benign or malignant pheochromocytomas of the adrenal gland were reported in male and female rats. Female rats were also reported to have alveolar/ bronchiolar adenoma and carcinoma of the lungs.

The authors⁵⁸⁷ concluded that there was “some evidence of carcinogenic activity” of talc in male F344/N rats based on an increased incidence of benign or malignant pheochromocytomas of the adrenal gland and “clear evidence of carcinogenic activity” in

female F344/N rats based on increased incidences of alveolar/bronchiolar adenomas and carcinomas of the lung and benign or malignant pheochromocytomas of the adrenal gland. “No evidence of carcinogenic activity” was reported in male or female B6C3F mice exposed to 6 or 18 mg/m³ of talc. Reviewers of the NTP-sponsored research commented on the increase in the spontaneous occurrence of pheochromocytomas in male rats in studies conducted over the prior 10 years⁵⁸⁸ and the fact that talc exposure does not provide a plausible mechanism for their increased occurrence since talc is relatively insoluble and it was unlikely that any soluble components could have reached concentrations high enough in the blood to affect the adrenal cells. Furthermore, it was noted that the way the substance is cleared by the lungs would make a direct effect on the adrenal gland very unlikely. The reviewers postulated that pheochromocytomas could be a nonspecific effect of stress as a result of chronic pulmonary inflammation. Few mice developed ovarian tumors; 2/38 (5%) of mice had an ovarian luteoma (a non-epithelial tumor) at 0 mg/m³ of talc and 0 mice had an ovarian tumor at higher doses. Only a few female rats developed non-epithelial ovarian tumors (1 (2%) developed a malignant granulosa cell tumor at 0 mg/m³; 2 (4%) developed benign granulosa cell tumors at 6 mg/m³; and 1 (2%) had a malignant granulosa-theca tumor at 18 mg/m³). The principal reviewers of the study noted that in light of the lung toxicity noted, the maximum tolerated dose was exceeded in female rats, and that the neoplasms noted were ‘irrelevant to human health risk assessment.’^{589,590} Additionally, the Danish Environmental Protection Agency⁵⁹¹ and the German MAK-Commission⁵⁹² attributed lung tumors in female rats to the general particle effect of granular biopersistent dusts, rather than talc particles. They also attributed the pheochromocytomas to an increase in cell proliferation due to hypoxia, which was considered to be a high-dose effect.

A study by Keskin and colleagues⁵⁹³ aimed to investigate whether long-term talc exposure is associated with potential carcinogenic effects on the female genital organs of 28 Sprague-Dawley rats. The experimental animals were allocated into four groups with seven rats each. Groups 1 and 2 served as controls; rats in Group 1 did not receive any intervention and Group 2 received intravaginal saline. Groups 3 and 4 received intravaginal or perineal talc application, respectively. Talc was applied for three months on a daily basis. Histopathological changes in the peritoneum and female genital system

were evaluated. In both groups exposed to talc (Groups 3 and 4), foreign body reaction and infection and an increase in inflammatory cells were found in the genital tissues. Genital infection was found in 12 rats in the study group and 2 rats in the control group. Neoplastic changes were *not* observed in the ovaries or peritoneum of the experimental or control groups. Although there was an increase in the number of follicles in animals exposed to talc, the authors did not attribute this change directly to talc exposure. Despite the study's main drawback that the animals were only exposed to talc for three months, talc was not shown to be carcinogenic in this study. Of note, Shim et al.⁵⁹⁴ also evaluated whole-body inhalation toxicity of talc, but ovaries were not a focus. Although domestic hens are known to have a markedly increased likelihood of developing ovarian adenocarcinomas,^{595,596} induced tumors in the laying hen model have not been used for evaluation of talc exposure.

Several animal studies have attempted to evaluate whether talc can migrate from the vagina to the ovary. Following intravaginal deposition of a talc suspension, talc particles were found in the ovaries of only two of six rats, while intrauterine deposition of the talc suspension resulted in talc particles being observed in the ovaries of eight rats.⁵⁹⁷ Because female cynomolgus monkeys resemble human females more than any other animal model in terms of physiological and anatomical parameters, translocation of particles from the vagina was evaluated in this species.⁵⁹⁸ Briefly, 0.3 ml of a 4% bone black suspension was deposited in the posterior vaginal fornix of each of five separately caged multiparous cynomolgus monkeys during mid-menstrual cycle. Each animal then received oxytocin. Oviducts of three animals were removed one hour after administration; the remaining two animals had oviducts removed 72 hours after administration. The oviducts were flushed with solution, which was subsequently collected in clean vials and filtered. Filters were examined for bone black particles by light microscopy, as were filters through which solution blanks (negative controls) were passed. Particles resembling bone black were identified on all filters (oviduct flush-filters and solution-black filters), likely due to contamination. The lab then conducted an experiment using two dosed monkeys and one control using suspensions of neutron-activated talc coupled with weekly injections of oxytocin. No measurable quantities greater than 0.5 micrograms of talc translocated from

the deposition site in the vagina to the uterine cavity or beyond after 30 consecutive days of douching with the suspension.

Animal models have also been used to assess vaginal irritation conferred by various agents.⁵⁸³ Currently, the preclinical test for the assessment of vaginal irritation required by the US Food and Drug Administration (FDA) for the regulation of spermicides and microbicides (regulated as drugs) and menstrual tampons and pads (regulated as devices) is the *in vivo* rabbit vaginal irritation (RVI) model because early reports found correlations between humans and rabbits regarding the irritation potential of vaginal formulations. Unfortunately, animals other than non-human primates, such as rabbits, are limited in their ability to mimic the human vaginal response due to differences in genital tract physiology and anatomy.⁵⁸³ As such, the field of toxicology is aiming to replace the use of animal models with alternative *in vitro* approaches, such as cell-based models (using cells from the genital tract) and explant-based or reconstructed tissue models.

Taken together, animal studies should be interpreted cautiously because of anatomic and physiological differences between animals and humans. Experimental models of rodents are particularly limited because ovulation in rodents occurs primarily during the breeding season, rodent ovaries are enclosed in an ovarian bursa and epithelial ovarian tumors are rare in these animals. Additionally, talc in animals is often administered at high doses with aerosol exposure, which certainly differs from the dose and mode of administration in humans. Furthermore, none of the animal studies directly examined potential transport of talc following external perineal administration. Finally, in most of the animal studies of talc, minimal to no characterization of mineralogy, fiber content, or particle size of samples was provided. Despite these limitations, I am unaware of animal studies that have shown that OvCa develops following direct talc injection.

In vitro investigations. Numerous *in vitro* studies have been performed to assess whether talc is a carcinogen, with many performed in the context of pleurodesis treatment for symptomatic pleural infections secondary to mesothelioma. Pleurodesis is a procedure used for patients with advanced lung, breast and other cancers that uses chemicals such as cosmetic-grade talc to seal up the space between the outer lining of the lung and the chest wall (pleural cavity) to prevent fluid or air from continually building up around the lungs. Patients with mesothelioma who have talc-induced pleurodesis can

have a lower morbidity than those who do not have pleurodesis, and long-term follow-up indicates that very few to no cases of lung cancer or mesothelioma have resulted from introduction of talc to the pleural cavity.⁵⁹⁹⁻⁶⁰¹ Additionally, patients with malignant pleural effusion (secondary to metastatic lung or breast cancer) treated with talc pleurodesis have been shown to have longer average survival times than patients in a control group treated with serial thoracentesis.⁶⁰² Thus, the fact that talc has been used for decades as a sclerosing agent for benign and malignant pleural effusions with favorable effects on quality of life and survival, suggests that talc, if anything, has an antitumor effect in the body, especially in the pleural space. Furthermore, data are lacking to support development of cancer because of the pleurodesis procedure. If talc actually led to cancer through inflammatory mechanisms, one would expect to observe a higher incidence of cancer among pleurodesis patients, a phenomenon that has not been observed.

One *in vitro* study⁵⁷⁸ was designed to evaluate whether talc directly affects cell death of malignant mesothelioma cells (MMC) or normal pleural mesothelial cells (PMC). Three confluent MMC and PMC were exposed to talc for 24, 48, and 72 hours, and glass beads of similar size to talc were used as controls. Apoptosis, or cell death, was determined by terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL) and DNA electrophoresis. Talc at a therapeutically achievable concentration (6 microg/cm²) induced significant apoptosis in MMC at 48 hours at a significantly greater level ($p < 0.05$) than in control cells. Talc did not lead to apoptosis in PMC, and glass beads did not cause significant apoptosis in MMC or PMC. This study suggested that talc induces apoptosis in MMC without affecting normal mesothelial cells of the pleura. This finding of selective apoptosis of lung cancer cells and sparing of normal mesothelium with talc (against other commonly administered intrapleural sclerosing agents such as bleomycin and doxycycline) has also been observed by other investigators.⁶⁰³ The lack of effect on normal cells, coupled with the induction of cell death in cancer cells, suggests that to the extent talc has any effect it inhibits cancer formation and development.

Angiostasis is a normal state that can be defined as the regulation by the body over the creation of new blood vessels, whereas angiogenesis or the state of generating new blood vessels only happens after injury or during tumor growth and progression.

Using proliferation, invasion, tube formation and apoptosis assays, talc was shown to alter the angiogenic balance in the pleural space from a biologically active and angiogenic environment to an angiostatic or stable milieu.⁵⁷⁷ Thus, talc acted in a protective role.

Importantly, talc has been shown to not be genotoxic in standard mutagenicity assays and has not been shown to induce mutations in any cancer-associated oncogene or tumor suppressor. *In vitro* response of rat pleural mesothelial cells to talc samples in genotoxicity assays previously developed for testing asbestos fibers (sister chromatid exchanges (SCE) and DNA repair) showed that none of the talc samples or negative controls induced enhancement of SCEs in treated compared to untreated cultures.⁶⁰⁴ Thus, talc does not induce mutations that are needed for cancer development and progression.

Another *in vitro* study evaluated whether talc could induce neoplastic change and, if so, whether talc-induced carcinogenesis in human ovarian cell cultures could be prevented by administration of pycnogenol (Pyc) (a proprietary mixture of bioflavonoids extracted from pine bark).⁶⁰⁵ In this experiment, the authors evaluated two *immortalized* human cell lines: ovarian surface epithelial cell line (OSE2a) and a granulosa ovarian cell line (GC1a). By virtue of being immortalized, they have been derived from sources such as tumors that harbor chromosomal abnormalities and mutations allowing them to be manipulated to proliferate and divide indefinitely to allow for culture for a long period of time. The authors call these “normal” cells, but they are not—they are prone to malignant transformation. Moreover, granulosa cells are not relevant to the study of epithelial OvCa. The cell lines used in this study were treated with talc or pretreated with Pyc then talc. Cell viability, reactive oxygen species (ROS) generation and neoplastic transformation by soft agar assay were measured. With regard to cell viability of talc treated OSE2a cells, a bell-shaped response was observed, with a statistically significant increase at 5 µg/mL (24 hours) and a statistically significant decrease at 200 µg/mL (72 h) and 500 µg/mL (24 hours and 72 hours). Similar trends were seen for the granulosa cells, with the number of transformed colonies decreasing significantly in the cells with the higher talc treatments (200 µg/mL and 500 µg/mL). The authors then report neoplastic transformation of cells by talc (compared to the untreated control); yet, the number of transformed colonies was actually reduced significantly with the highest dose (100 µg/mL) for the OSE2 cells.

Finally, even though the authors report that talc increased ROS generation, the data do not support that. Instead, decreases in ROS generation seem to have been observed for both cell lines at increasing doses of talc. Pre-treatment with Pyc did not cause significant changes in cell viability or neoplastic transformation of the OSE2 cells. Based on these collective findings, especially those related to a lack of a dose-response trend in OSE2 cells and the fact that the authors did not show that the generation of ROS following talc exposure led to cell damage or genetic alterations, I *disagree* with the authors' interpretation⁶⁰⁵ and that of Taher et al.⁵⁰⁵ that this study supports talc as a contributor to ovarian neoplastic transformation. Additional experiments would be needed to gain further support for that argument.

Over the last several years, a team from Wayne State University (led by a senior author who serves as a paid consultant and expert witness for plaintiffs in the talcum powder litigation) have conducted various experiments on talc, funded in part by plaintiffs' counsel. The first such paper looked at research on the role of oxidative stress in the pathogenesis and dissemination of OvCa.^{606,607} The authors sought to determine the effects of talcum powder on the expression of key antioxidant and antioxidant enzymes, CA-125 levels and cell proliferation and apoptosis in EOC cells and normal epithelial cells.⁶⁰⁸ Human EOC cells (SKOV-3, TOV112D, A2780, and OV90) and human primary normal epithelial cells were used for analysis. Of note, although SKOV-3 and A2780 are frequently used to represent high-grade serous EOC, they are poor models of HGSOE due to the lack of *TP53* mutations and flat copy number profile.⁶⁰⁹ The authors' early 2019 publication⁶⁰⁸ reports that all cells were treated with 5, 20, or 100 µg/ml of talc (Johnson's Baby Powder) for 72 hours, which differs from the dosage (0, 200, 500 and 1000 µg/ml) and timing (additional measurements at 24 and 48 hours) reported by the same authors in abstract submissions from 2018 on this same content.^{610,611} Sterile dimethyl sulfoxide (DMSO) was reportedly used as a control for all experiments. Using real-time reverse transcription polymerase chain reaction and enzyme-linked immunosorbent assays, levels of CA-125, caspase-3, nitrate/nitrite, and selected key redox enzymes, including myeloperoxidase (MPO), inducible nitric oxide synthase (iNOS), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and glutathione reductase (GSR), were measured before and after 72 hours of talc treatment. Single-nucleotide

polymorphisms in genes corresponding to target enzymes were identified using TaqMan genotype analysis. Cell proliferation was determined by MTT proliferation assay and apoptosis was determined using the caspase-3 colorimetric assay.

The authors reported an increase in messenger RNA (mRNA) levels of pro-oxidant enzymes, iNOS and MPO in talc-treated OvCa and normal ovarian epithelial cells, compared to controls, in all doses (5, 20 and 100 µg/ml) and reported a dose response.⁶⁰⁸ However, a clear dose response with a higher dosage of talc treatment does not appear to be present for iNOS in the SKOV3 and TOV112D EOC cell lines (Figure 2A) or for NO₂/NO₃ activity for the TOV11D cell line (Figure 2C).⁶⁰⁸ Furthermore, the expression of MPO appears to actually be higher in the control for SKOV-3 compared to the 5 µg/ml talc-treated SKOV-3 cell line (Figure 2B), and MPO activity was higher in controls for normal ovarian and fallopian tube cells compared to the 5 µg/ml talc treatment group cells.⁶⁰⁸ In their article, the authors reported a decrease in mRNA levels of antioxidants CAT, SOD3, GPX and GSR in talc-treated OvCa and normal ovarian epithelial cells, compared to controls, in all doses; yet, their prior abstract⁶¹² says an increase in GSR and no change in GST was observed. Furthermore, it appears from Figure 3B that there was an *increase* in the expression of GPX in the TOV112 cell line between the 20 ug/ml and 100 µg/ml dose.⁶⁰⁸ The authors claimed that talc exposure induced specific point mutations that are known to alter the activity in some of these key enzymes, but it is unclear how exactly this would occur since such DNA damage would be random and there were no positive controls used. Moreover, it is unclear why point mutations are considered, since HGSOC is a disease of copy number alterations, not point mutations. The Saed team claims to have shown that specific point mutations in key redox enzymes can be introduced into human EOC cell lines and their chemoresistant counterparts using the CRISPR/Cas9 technique;⁶⁰⁷ it is suspected that a similar technique may have been introduced here and/or these SNPs may be inherent to the cell line(s) used. Talc exposure also was reported to result in a significant increase in tumor marker CA-125 levels in all cells ($P < .05$) and to induce cell proliferation and decrease apoptosis in cancer cells and to a greater degree in normal cells ($P < 0.05$).

The authors⁶⁰⁸ claim that they have “shown beyond doubt that talc alters key redox and inflammatory markers, enhances cell proliferation, and inhibits apoptosis” and claim

that they have “confirm[ed] the cellular effect of talc and provid[ed] a molecular mechanism to previous reports linking genital use to increased ovarian cancer risk.” In my opinion, these are very strong and unfounded claims. The study (and those that followed) lacked a discussion of how CA-125 and levels of key redox enzymes can be linked to inflammation, much less to the oncogenic processes. They also failed to include positive controls (i.e., a control group that uses a treatment that is known to produce results of interest). Additionally, caution should be used when interpreting these findings because they derive from four cell lines that are not necessarily representative of most human ovarian cancers. Analyses of human tissues may have been more informative. There were also inconsistencies found within their publication and between the publication and prior recently submitted abstracts. Certainly, results can evolve with further experimentation, but there were inconsistencies in the design, raising concern about the integrity of the findings and their interpretation. Furthermore, discussion was lacking regarding the relevance of *in vitro* talc concentrations to those used in humans. Additionally, it is of interest that the findings of this team⁶⁰⁸ are contrary to a study of rats that observed increased expression of the antioxidants GSR and SOD1 in the talc-treated group.⁶¹³ Saed and colleagues also do not provide data to support the oxidation state of pre-malignant lesions in which a purported carcinogen suspected to promote inflammation would be expected to play a role.⁶⁰⁸

Noteworthy is that prior to publication, this paper⁶⁰⁸ was previously rejected by the journal *Gynecologic Oncology* on 9/19/2018⁶¹⁴. The *in vitro* results were described as “not sufficiently convincing,” and the reviewers stated that their⁶⁰⁸ data “do not show, despite the authors’ claim, any evidence that these cells are transformed. Specifically, no experiments documenting changes in cell survival, proliferation or resistance to apoptosis have been performed. Consequently, neither tumor initiation nor progression is documented in this study. While changes in redox potential play an important role in tumor biology in general, the present data are insufficient to back up the claim that talcum is central to the development of ovarian cancer.”

More recently, the same authors claimed (erroneously) to have shown that talcum powder induces malignant transformation in human primary normal ovarian epithelial cells.⁶¹⁵ The authors’ manuscript⁶¹⁵ was rejected by several journals, including

*Gynecologic Oncology*⁶¹⁶, *PLOS One*⁶¹⁷ and *Reproductive Sciences*⁶¹⁸, prior to being published in a low-tier Italian journal. Major concerns raised by peerreviewers for the journals included the following: (1) reliance on a single commercial assay (an in vitro anchorage-independent growth assay) for assessment of transformation; colony formation does *not* signify a molecular mechanism of action as the authors suggest when misleadingly stating that “this finding represents a direct causation mechanism of talcum powder exposure”; (2) appropriate statistical tests were not applied, and therefore data are challenging to interpret; (3) the questionable clinical relevance given the arbitrary high-dose selection of talcum powder; (4) ovarian surface epithelial cells were examined without comparison to fallopian tube secretory epithelium, the probable origin of HGSOC; and (5) correlation to immunohistochemistry (for markers Ki67 and p53) is inadequate for evaluating functional changes in cells suggestive of malignant transformation. In fact, in the rejection from PLoS One⁶¹⁷ on 10/28/2020, the editor noted that, “both reviewers have raised serious concerns about the experimental design, analyses, and interpretation of findings.” Months later, after the authors resubmitted the paper to *Gynecologic Oncology*,⁶¹⁶ the editor of that journal stated the following on 2/2/2021: “Of primary concern is reliance on a single commercial assay for assessment of transformation that has not been established in the literature. Moreover, statistical tests were not applied and thus the data are hard to interpret. The clinical relevance is questionable given the arbitrary dose selection of talcum powder, and perhaps more importantly the examination of ovarian surface epithelial cells without comparison to fallopian tube secretory epithelium. Given that the prevailing evidence suggest the origin of high grade serous ovarian cancer is the fallopian tube, the data presented are of limited relevance.”

A study by Shukla et al.⁶¹⁹ was conducted to compare the acute toxicity and gene expression profiles of equal surface area concentrations of crocidolite asbestos, nonfibrous talc, fine titanium dioxide (TiO₂), and glass beads in a human mesothelial cell line (LP9/TERT-1) and in human ovarian epithelial cells (IOSE) cells.⁶¹⁹ Cell lines were exposed to low (15 $\mu\text{m}^2/\text{cm}^2$) and high (75 $\mu\text{m}^2/\text{cm}^2$) concentrations of each mineral for 8 or 24 hours; cell viability was assessed using trypan blue exclusion tests; and the gene expression profile was evaluated by microarray and quantitative real-time polymerase chain reaction analysis. High concentrations of asbestos were found to be significantly

more toxic in LP9/TERT-1 cells than in untreated cells, while high doses of talc appeared nontoxic. Asbestos-induced gene expression changes in LP9 cells appeared to be concentration and time-dependent, with fewer gene expression alterations at low concentrations of asbestos than at high concentrations, at 29 and 239 significant alterations, respectively. Most deregulated genes were proinflammatory cytokines. Furthermore, the number of significant gene alterations increased over time in the asbestos-exposed LP9 cell line. On the other hand, few gene expression changes (n=30) were observed at high concentrations of talc in the L9 cells, and no gene expression changes were observed with low concentrations of talc or at any concentration or timepoint with TiO₂ or glass beads. IOSE cells showed very few gene expression changes in response to asbestos, and no significant gene changes were observed with talc, TiO₂ or glass beads. It is important to note that this study examines a cell type (immortalized pleural mesothelial cells) that is not directly relevant to OvCa pathogenesis. Furthermore, the changes in gene expression are very slight. Also, IOSE are not likely to be the target cells for most HGSOE, and there is no evidence that talc causes anything other than granulomas, which are not typically associated with OvCa. Taken together, only alterations in gene expression and cytokine/chemokine changes induced by crocidolite asbestos in LP9/TERT-1 cells may be indicative of its increased potential to cause mesothelioma in comparison to the other nonfibrous materials examined.

Studies of Human Tissues. Using an extraction-replication technique to examine asbestos within the tissue of various mesotheliomas, Henderson (1971)⁶²⁰ examined multiple sections of ovarian tumors. No asbestos particles were found, but talc particulates were observed in 10 of 13 ovarian tumors examined, 12/21 cervical tumors, and 5/12 normal ovaries. No correlation was identified between particle count and patient-reported talc exposure. This finding was “greeted with skepticism” because of possible contamination by talc on surgical gloves at the time of specimen collection and processing.⁶²¹ A re-evaluation of nine new specimens collected from deep within tissue samples extracted with the use of forceps and “noncontaminated” surgical gloves reported the presence of talc particulates in all nine specimens, including ovaries free of disease.⁶²²

In 1996, Heller and colleagues⁵⁷⁹ conducted the first pathoepidemiologic investigation to correlate self-reported history of perineal talc usage with talc particle burden found in ovaries. Ovaries were studied from 24 women undergoing incidental oophorectomy (at Columbia Presbyterian Medical Center) who were interviewed for talc usage. To simplify the classification of exposed and unexposed women, subjects who reported tubal ligation, diaphragm use, or feminine hygiene spray were excluded from analyses. Therefore, the “exposed” group only included women who reported talc application to undergarments or to the perineum. Sections of normal ovary from the two women who reported the largest number of talc applications were analyzed, and the unexposed women closest in age were chosen as controls. Talc particle counts were examined in ovarian specimens from the 24 women (12 who reported frequent perineal talc applications and 12 who reported no use but were closest in age) and compared to the reported frequency and duration of talc use. Ovarian tissue blocks were digested and analyzed by polarized light microscopy and analytic electron microscopy to identify and quantify talc. Talc was detected in all ovaries by polarized light or electron microscopy and observed to a similar extent for exposed and unexposed subjects. No relationship was found between talc burden in healthy ovarian tissue and lifelong talc dusting. Importantly, no evidence of a response to talc was observed in the tissue, such as foreign body giant cell reactions or fibrosis. Asbestos was detected in the ovaries of five subjects with no talc exposure and in four ovaries of talc-exposed subjects. If transvaginal transport of perineally applied talc occurs, we would expect women with the heaviest exposures to show the largest talc particle burden in their ovaries. Results *do not* support a linear dose-related ovarian talc particle burden. Mean electron microscopy count was higher in users vs non-users; however, other factors that may contribute include method of application, type of talc, and possible contribution of inhaled talc particles. Talc exposure from examination and surgical gloves is also a possibility.

In humans, two studies observed migration of inert carbon particles⁶²³ and radioactively labeled albumin microspheres⁶²⁴ from the vagina to the fallopian tubes. In one of those studies, Egli and Newton⁶²³ placed inert dextran particles via a speculum into the vagina of three women undergoing elective hysterectomy who were under general anesthesia, administered oxytocin, placed the women in the supine, flat position,

retrieved the fallopian tubes, and identified particles in 2 of the 3 specimens within approximately 30 minutes. It is worth noting that this study has a very small sample size, and its design does not remotely resemble the typical manner that a woman would dust talc in the perineal area. Moreover, information is lacking regarding the dose of the particles administered versus that which may be expected from perineal dusting. The authors contend that the oxytocin administration is physiologically relevant since oxytocin is released during intercourse. If that were true, one would expect that talc on diaphragms or on condoms would show the greatest association with OvCa risk in epidemiological studies. However, as described earlier in this report, epidemiological studies have observed the opposite. Additionally, some have observed birefringent crystals embedded in ovarian tissue,^{620,625} but these studies were poorly designed and used imprecise methods to measure particulates due to leaching of radionucleotide markers from the test materials or introduction of contaminants during processing.⁵⁹⁸ In another study, Venter and Iturralde⁶²⁴ deposited radioactively labelled albumin microspheres into the posterior fornices of 24 patients the day prior to various procedures. Only 21 cases could be evaluated. Of these, 16 showed radioactivity reaching the uterus or higher. Again, this study suffers from a lack of relevance between depositing large numbers of particles in the posterior fornix (internally near the cervix) versus external perineal dusting of talc.

A study in humans was performed to determine whether starch particles from powdered gloves may gain access to the abdominal cavity through the vagina after routine gynecological examination prior to an elective laparotomy or hysterectomy.⁶²⁶ Peritoneal fluid was collected after the procedure and cell smears of the fluid (and cervix and uterine tissue and fallopian tubes, if removed) were taken and analyzed quantitatively. No ovarian samples were evaluated. The authors reported more starch particles in cell smears from patients examined with powdered gloves, although in two patients examined with powdered gloves, no particles were found, and in three patients examined with powder-free gloves, a few particles were identified. Lower numbers of particles were observed among women examined four days preoperatively. The authors suggested that starch particles may migrate from the vagina into the cervical canal, uterine cavity and through the fallopian tube. Again, however, this study involved particles deposited (sometimes with force) well into the internal genitalia.

In short, as reiterated by Goodman et al.,⁵⁷⁴ “no identified studies of humans or animals specifically examined particle transport following external application to the perineal area. Instead, the studied particles were typically in a solution and placed within the reproductive tract (e.g., intravaginally).

In 2019, an investigative team led by first author, Dr. McDonald, published on several laboratory investigations of talc using human tissues; co-authors include Dr. Daniel Cramer and several others who provide expert testimony on behalf of the plaintiffs in talc litigations).^{627,628} Both of these studies by McDonald and colleagues^{627,628} evaluate the hypothesis that talc may gain access to the lymphatic system as a means of reaching pelvic organs and lymph nodes. In the first investigation,⁶²⁷ the authors expanded upon a prior case report⁶²⁹ and set out to differentiate whether talc found in lymph nodes was due to exposure or contamination by studying only lymph nodes (and not ovaries) from 22 women with various types of ovarian tumors (20 malignant (14 serous histology), 1 borderline, and 1 granulosa cell) who presented for care in 2004 and 2005 at Brigham and Women’s Hospital. Ten of the 22 women had self-reported ever use of talc in their genital area; duration and/or frequency of talc use was not reported in this study. The authors⁶²⁷ also evaluated a second group of 19 lymph node specimens from 10 ovarian carcinoma cases, which were consults of the authors. They⁶²⁷ quantified talc using digestion of tissue taken from paraffin blocks and scanning electron microscopy/energy dispersive X-ray analysis (SEM/EDX), and compared the SEM/EDX data to findings obtained by polarizing light microscopy. Although talc particles were found in some digested lymph nodes, they correlated with contamination, likely from non-surgical gloves. In addition to talc, other birefringent materials were identified, such as magnesium and aluminum silicates. Such nonfibrous, non-talc silicates are known to have a longer dissolution time than talc. In the second part of their study of lymph nodes from 19 cases, the authors showed that surface contamination particles included talc and other exogenous particles. The authors⁶²⁷ concluded that talc contamination of the surface of surgical pathology specimens is common and that correlative light microscopy is needed to assess the possibility of lab contamination, and to determine whether talc is present in lymph nodes or other tissues.

McDonald and colleagues⁶²⁸ also examined tissue in multiple pelvic organ sites from five cases with self-reported perineal talc use (and six negative exposure control patients), using polarized light and scanning electron microscopy. It is worth noting that the five patients chosen for this analysis were received for “consultation” by some of the authors over a three- to four-year period and were newly diagnosed, whereas the control patients were selected from the large New England Case Control Study and had been diagnosed many years prior. Furthermore, the five cases with reported talc exposure seemed to have been chosen (out of a larger set of 34 cases seen for consult) because they had more than 30 years of talc exposure. The distribution of established and suspected OvCa risk factors was not provided for the exposed or unexposed cases. Talc was found in two pelvic organ sites for three patients, in three sites for one patient and in four sites in the final patient. Four talc particles were found in two patients who underwent pelvic surgery more than 30 years prior. Foreign non-talc particles were also detected and included silicon oxide and metals such as copper and magnesium. The authors⁶²⁸ postulated that talc may access the lymphatic system directly in the perineum or at any point in its ascent through the genitourinary tract toward the fallopian tubes and ovaries. *If* indeed talc does migrate through the lymphatic system to the genitourinary tract and *if* talc were to have a role in contributing to cancer, we might expect to see histologic changes or cancer in the pelvic sites sampled. The fact that signs of fibrosis or cancer were *not* observed at these sites provides additional evidence that talc does not cause cancer in pelvic organ sites.

In a subsequent laboratory study published by members of the same team in 2020, Johnson and colleagues⁶³⁰ compared talc particles from commercially available powders to those found in pelvic tissues obtained from 11 randomly-selected OvCa patients with reported long-term self-reported perineal talc use. These 11 cases were sent for consultation services by the senior author, John J. Godleski (JJG); Godleski and first author, Kurt Johnson, are paid Plaintiff expert witnesses. It is noteworthy that no details were provided regarding the institutions where the women had their surgeries, the risk factor profile of the women, or the exposure history to talc in terms of duration and/or frequency. PLM and SEM/EDX were employed to measure the talc particles, and the authors reported that they controlled for contamination. The authors⁶³⁰ show that the talc

particles taken from tissues of the patients were most often located within benign tissue, reactive fibroblastic tissue or chronically inflamed tissue near a tumor *instead* of within tumors. They then stated that “talc does not need to be found within the actual tumor tissue to support a causal link, the presumption being that it accumulates in benign ovarian or other female genital tract tissue some time before tumor develops.” I find this logic hard to follow and in no way does their⁶³⁰ work support the theory that talc can contribute to the development of OvCa. Thus, I disagree with Plaintiff’s expert witness Dr. Singh⁵⁷³ that “Johnson et al.⁶³⁰ provided additional proof of retrograde migration of talc particles.” I also find it concerning that they continue to point to methodologically flawed *in vitro* work by the Saed group⁶⁰⁸ to attempt to show talc as a factor contributing to carcinogenesis.

In 2020, Mandarino and colleagues⁶³¹ attempted to evaluate the effects of talc (compared to control particles, which included titanium dioxide, concentrated urban air particulates or diesel exhaust particles) on the ability of macrophage cell lines to slow the growth of murine ovarian surface epithelial cells (MOSEC) in culture in the presence of estradiol. The authors reported that coexposure of macrophages to talc and estradiol led to increased production of reactive oxygen species and changes in gene expression. However, the study had several design limitations that preclude the ability to draw any strong conclusions: cosmetic talc products were not used in any experiments—rather, high and seemingly unrealistic concentrations of talc and estradiol were used; dose-response toxicity analysis failed to reveal significant toxicity to macrophages using talc alone or in combination with estradiol; the expression profiling that was undertaken was not comprehensive and did not evaluate whether the changes were unique to talc; the effects of talc and estradiol as shown appear to be additive, which does not support a novel mechanism of immune suppression; and finally, it is unclear how closely phagocytic murine cell lines and MOSECs resemble human ovarian cancer cells. In a more recent study by this same team, Emi and colleagues⁶³² published a report on the transcriptomic and epigenomic effects of non-commercially available talc and titanium dioxide particles on murine J774 macrophage cell lines. They identified shared transcriptomic and epigenomic changes between talc and titanium dioxide, but also noted a specific pattern of gene expression and DNA methylation changes for each particle type, especially when

estrogen was added. Similar concerns as highlighted above for their earlier publication⁶³¹ are noted, especially in light of the important differences that exist between primary macrophages and cell lines (from murine models) that need to be accounted for when choosing a macrophage model to study host-exposure interactions^{633,634}.

In terms of other studies that evaluated a link with macrophages, Bogatu and Contag⁶³⁵ have suggested that talc ingestion may induce proinflammatory effects on macrophages by binding to high-density lipoprotein particles. Some plaintiffs' experts argue that this may trigger a fibrogenic reaction/scar formation. However, data are lacking to support involvement of scarring/fibrosis in ovarian carcinogenesis, and talc has not been identified in ovarian scar tissue. Davies et al.⁶³⁶ also studied the effects of respirable talc dust on mouse peritoneal macrophages, and reportedly observed mild cytotoxicity, and suggested talc could be fibrogenic. However, again, there is no evidence that fibrosis plays a role in ovarian cancer pathogenesis. Hamilton et al.⁶³⁷ reported that talc caused a small increase in the proliferation of mouse bone marrow-derived macrophages and argued that talc could cause granulomas. As noted in this report, granulomas have been identified in prior talc injection studies, but it is worth noting that granulomas are not precursors to OvCa. Akhtar et al.⁶³⁸ measured the effects of talc on A549 cells, and reported ROS production, oxidation of cellular lipids and DNA damage. However, the relevance of this work to ovarian carcinogenesis is limited since A549 are lung cancer cells, and it is unclear how or why the doses used relate to particles in the female reproductive tract.

Taken together, these lines of *in vitro* and *in vivo* work are highly speculative, may not be representative of processes in humans, and do not provide evidence that talc induces ovarian carcinogenesis.

III. ASSESSMENT OF CAUSALITY

A. Introduction to Bradford Hill Guidelines

In evaluating epidemiologic studies, it is critical to emphasize that associations are *not* equivalent to causation; statistically significant results may not be biologically meaningful or methodologically appropriate for contributing to causal inference. Determining whether a statistical association is causal involves numerous considerations. As shown in **Figure 1**, a statistical association between an exposure and an outcome can

be spurious or false (due to random error or bias), non-causal (due to confounding by an extraneous factor or because an outcome caused the exposure rather than the exposure causing the outcome) or causal (due to a cause-effect relationship between exposure and disease).⁵⁰¹ Since it is not possible to prove causation directly with observational studies, it is helpful to have reliable guidelines upon which to judge a statistical association in terms of its likelihood to be causal. When most or all guidelines are met, the likelihood of causality increases. The scientific community applies guidelines for assessing causality, which are known as the Bradford Hill considerations or guidelines,⁶³⁹ when evaluating associations between an exposure and a particular disease or outcome to arrive at conclusions and opinions regarding causality. A total of nine Bradford Hill guidelines exist, and I considered all of them in developing my assessment. However, the six featured in **Figure 1** are thought to be most important in assessing causality: correct temporal sequence, strength of association, consistency of the association, dose-response relationship, biological plausibility and experimental evidence. All nine Bradford Hill guidelines⁶³⁹ are summarized below:

1) Temporality (i.e., did the exposure precede the outcome?). This guideline is essential when assessing whether an association is causal. Genital talc exposure would need to *precede* a diagnosis of cancer to be causal. In a prospective cohort study (or in a “nested” case-control study), exposure status is assessed before disease status, and therefore satisfies the consideration of temporality. However, in traditional case-control studies, exposure status is based on historical recall over a person’s lifetime.

Factors that must be considered when evaluating causality include the induction period and the latency period. The induction period is the time it takes for the causal agent or exposure to initiate the disease process, and the latency period is the time it takes from disease initiation to when the disease is clinically diagnosed due to overt signs or symptoms. For chronic diseases like cancer, immediate follow-up of the exposed group can result in non-differential misclassification with bias of the measure of association toward the null value. This can occur because subjects in the exposed group are not technically “at risk” of manifesting the outcome due to the study exposure until a specific “induction period” has transpired. OvCa takes years to develop and therefore is likely to occur many years after exposure to risk factors. Thus, the pertinent follow-up period for

exposed subjects should correspond to the expected latency period for the outcome based on the exposure, since exposed subjects are not considered at risk of manifesting the outcome until after the induction period has occurred. **Table 1** summarizes selected characteristics of key case-control and prospective cohort studies that evaluated the talc-OvCa risk association.

Sometimes, talc exposure may have occurred *concurrently with or subsequent to* an OvCa diagnosis, and therefore would not have altered the frequency of disease occurrence. As an example, Huncharek et al.⁴¹⁴ suggested that population-based cases may spuriously show an association secondary to an “exposure misclassification” or a “treatment effect” because some women with OvCa will undergo treatment (with radiation, chemotherapy and/or surgery), which leads to side effects such as irritation. Because talc keeps skin folds in the perineum (the area between the vagina and the anus) dry and prevents skin breakdown secondary to treatment, this could prompt talc use. Thus, patients may not always make the distinction between pre-diagnosis and post-treatment use. Exposure misclassification among prevalent cases may cause a spurious finding of an association when none in fact exists.

2) Strength or Magnitude of the Association (i.e., is the association strong and statistically significant?). The stronger an association, the less likely it is that the association reflects the influence of some other etiologic factors or other methodological errors. A “significant” exposure-disease association exists when the two occur together more frequently than they would by random chance. Once an association is observed, the strength of the association is examined and assessed for the presence of bias and/or confounding. In case-control studies, an “odds ratio (OR)” is typically estimated. An OR represents the ratio of the odds of exposure among the cases to the odds of exposure among the controls (exposure odds) or the odds of the outcome among the exposed to the odds of the outcome among the unexposed (disease odds). Prospective cohort studies produce a “relative risk (RR) or risk ratio,” which is calculated by taking the ratio of the risk of an occurrence among those exposed to a certain agent to those who are not exposed to that agent. (Of note, the exposure-odds ratio calculated in case-control studies can mathematically approach the RR as the prevalence of the outcome falls below 10%, which is the situation for a rare disease like OvCa, with a prevalence of less than

2%.) An OR or RR below 3.0 is considered weak or moderate.⁶⁴⁰⁻⁶⁴² As will be pointed out in this report, among case-control studies that reported positive associations between ever use of talc in the perineal area and OvCa risk, many were *not* statistically significant upon review^{337,467,502,509,510,516,522,525} (**Table 1**). Of statistically significant weak positive associations that were detected,^{40,271,307,506,512,513,517,518,520,523,526,527,531} most had ORs or RRs of 1.2-1.3, i.e., “weak” associations (**Table 1**). Thus, by no means have existing studies that evaluated positive associations between talc and OvCa risk shown strong associations.

3) Consistency of the relationship (i.e., whether numerous epidemiologic studies, using a variety of locations, populations and methods, repeatedly observe a similar association between an exposure and an outcome with respect to the null hypothesis of no association). The criterion of consistency helps to protect against associations arising due to error or artifact. Among the case-control studies that assessed whether there is an association between talc and OvCa, some reported a positive association,^{40,271,307,506,512,513,517,518,520,523,526,527,531} and others reported no association,^{414,498,507,508,510,514,516,522,542,643,644} demonstrating inconsistency (**Table 1**). Moreover, inconsistency was observed when comparing findings from population-based studies (which tended to observe weak statistically significant associations) with hospital-based studies (which did *not* tend to observe statistically significant associations) (**Table 1**). Inconsistency has also been observed when comparing findings from case-control and prospective cohort studies, with none of the prospective cohort studies^{31,497-500} reporting a positive association between talc and OvCa overall. Furthermore, when statistical tests of heterogeneity were conducted as part of meta-analytic or pooled approaches, great heterogeneity was observed between case-control studies, which should have precluded them from being combined together.^{413,502,505} On the other hand, homogeneity was observed when comparing the prospective cohort studies, suggesting it was appropriate to combine the data⁵⁰⁰ (**Table 2**). Most studies of perineal exposure and OvCa risk took place in the US,^{31,40,467,497,498,507-509,511,512,515-518,520-523,526,527,542,548,556} but other countries were also represented, including Canada,^{513,514} Australia,^{140,271,307} Norway,⁴⁶⁶ China,¹⁷⁰ Greece⁵¹⁰ and the United Kingdom,¹³⁶ with presence and absence of associations reported in many of these locations (**Table 1**). Studies that reported positive associations

were carried out between 1982 and 2016, as were studies that reported an absence of an association (**Table 1**). Thus, inconsistency exists within and between study designs, locations and time periods. It is important to note that while several recent meta-analyses (**Table 2**) have calculated similar weak magnitudes of effect and had similar conclusions, this apparent “consistency” results from the fact that they largely considered the same component studies. Finally, when integrating data, knowledge and reasoning from multiple disciplines and approaches (including epidemiology, molecular biology, genetics, toxicology and statistics) to assess a possible association between talc and OvCa, evidence is *lacking* across disciplines to support a consistent (and biologically plausible) association.

4) Dose-Response Relationship/Biological Gradient (i.e., is a dose-response observed with increasing levels of the exposure?). Dose-response curves can vary from one study to the next depending on the unique characteristics of the population, exposure routes and endpoints assessed. Synergistic or antagonistic effects of cumulative exposures can make some biological gradients even harder to characterize.⁵⁷⁵ As noted above, there are inherent limitations in quantifying a dose-response relationship with talc due to a lack of metrics for how much talc is in an application, how much enters the vagina, and how much possibly reaches the upper reproductive tract, if any.

Ultimately, compelling data are lacking to support a dose-response relationship between talc use and OvCa risk. In the most recent pooled analysis of prospective cohort studies, the authors reported no dose response.⁵⁰⁰ In a recently published systematic review by Taher et al.,⁵⁰⁵ several studies^{467,509,520,521,523,527} with a broad range of sample sizes (i.e., between 77 and 2,041 OvCa cases) were reported to have shown a dose-response relationship. However, upon looking closely at the data generated by the component studies, clear trends of statistically significant dose responses were *not* present in several of these studies^{509,520,521,523} (**Table 3**). Importantly, of numerous studies that evaluated dose-response relationships by frequency and/or duration of talc use, no clear trend was found,^{136,271,307,337,497,512,513,516-518,525,542} despite erroneous interpretations of “significant trends” for a few studies^{467,509,513,523} in Taher’s meta-analysis⁵⁰⁵ (**Table 3**).

If a dose-response relationship is observed, it is important to evaluate whether symptoms of the disease process itself could influence use of an agent or exposure, as

was pointed out by Huncharek et al.⁴¹⁴ (i.e., irritation from treatment may have caused some women to use talc to counter symptoms). It is also worthwhile to note that advances in our understanding of genetic polymorphisms/variants may underscore reasons behind individual variations in biological responses to exposures. This is a concept Gates et al.⁵²⁰ attempted to illustrate when evaluating interactions between talc use and variants in genes involved in detoxification pathways. Only weak associations were reported, and those associations did not provide much insight into a relationship between the candidate polymorphisms, talc exposure and OvCa risk. In conclusion, if talc had pro-oncogenic effects, one would expect to observe an increasing effect with increasing doses. However, each of the prospective cohort studies, numerous case-control studies, several meta-analyses and pooled studies have failed to show a clear and consistent dose-response relationship (**Table 3**).

5) Biological Plausibility (i.e., is there a biologically plausible mechanism whereby the agent/exposure could cause the disease?). This consideration is based on the scientific recognition that epidemiologic associations must have a biological explanation to be causal. Therefore, we must ask ourselves whether the relationship is consistent with the current body of knowledge regarding the etiology and mechanism of disease. Biological plausibility can be examined with *in vivo* and *in vitro* experiments that evaluate defined disease mechanisms. Multiple lines of evidence from epidemiology and biology do *not* support a biologically plausible argument for how or why talc could cause OvCa, which is an insurmountable flaw in the talc-OvCa hypothesis. Specifically, available publications addressing a potential talc-OvCa risk association do not provide a rational means by which talc can travel to the ovaries; nor do they show compelling evidence of talc causing an inflammatory response in ovarian cells.

With regard to the mechanism by which inanimate talc particles could migrate from the perineal area to the ovaries, the hypothesis purported by Cramer et al.^{506,548} suggests that the talc must migrate upwards against gravity, the downward flow of vaginal mucosa and menstrual fluids and the cilia of the fallopian tube epithelium. This hypothesized process is not mechanistically logical. And, as discussed above, to the extent some experimental findings support the possibility of such retrograde migration to the tubes or ovaries, they involve circumstances completely different from external perineal use.

Furthermore, if talc particles supposedly migrate from the perineum to the ovaries and promote cancer, one would expect the talc particles to lodge elsewhere along the reproductive tract and promote cancer development there also. I have not come across compelling data to support an increase in other gynecological cancers, such as vaginal, cervical or fallopian tube cancer with talc use. Some studies have detected associations between talc and endometrial/ uterine cancer risk, with inconsistent findings.⁶⁴⁵⁻⁶⁴⁸ Although one prospective study of the NHS Cohort by Karageorgi et al.⁶⁴⁶ supports a very weak association (RR=1.21 (95% CI:1.02, 1.44)) between ever use of talc in the perineal area and endometrial cancer risk, two other studies (one prospective cohort involving the Women's Health Initiative Observational Study (WHI-OS)⁶⁴⁵ and one population based case-control study of the Australian National Endometrial Cancer Study (ANECs))⁶⁴⁷ showed that in general, perineal talc use is *not* associated with endometrial cancer risk. Use of powder on a diaphragm for 20 or more years was associated with a statistically significant increased risk for endometrial cancer, while use of talc on a diaphragm for <20 years was not associated with an increased endometrial cancer risk.⁶⁴⁵ In 2019, O'Brien et al.⁶⁴⁸ examined the relationship between self-reported use of talc or douche and incident uterine cancer in a large prospective cohort study. Twenty-six percent of women reported ever using talc, and 15% reported ever having douched. Ever use of talc was associated with an increased but not statistically significant risk of uterine cancer (adjusted hazard ratio [HR] = 1.2, 95% CI: 0.94, 1.6), with no significant dose-response relationship observed for frequency of talc use (P-for-trend = 0.07). Ever douching was not associated with uterine cancer risk (HR = 1.0, 95% CI = 0.72, 1.5), and a dose-response relationship was not observed (P = 0.96). Additionally, a recent pooled analysis evaluated the association between genital powder use and uterine cancer risk using updated data from three prior cohort studies plus NHSII; results were null (HR=1.1, 95% CI: 0.94-1.09).⁶⁴⁹ Finally, O'Brien et al. recently evaluated the association between douching, genital talc use and the risk of cervical cancer in the Sister Study.⁶⁵⁰ The authors did not detect an association between adolescent talc use and pre-baseline cervical cancer (HR 0.95, 95% CI 0.76–1.19), but douching in the year prior to enrollment was positively associated with incident cervical cancer (HR 2.56, 95% CI 1.10–5.99). The association between recent genital talc use and incident cervical cancer was not

statistically significant (HR 1.79, 95% CI 0.78–4.11). Finally, Cramer and colleagues^{627,628} have recently suggested that talc may gain access to the lymphatic system as a means of reaching pelvic organs and lymph nodes, but there is no evidence to support this hypothesis.

Assuming that talc could migrate to the tubes or ovaries, Cramer et al. and other groups^{271,506,548,651} proposed the inflammatory hypothesis to explain how talc may contribute to OvCa development. The inflammatory hypothesis suggests that talc flows upstream, lodges in the ovaries, irritates ovarian cells, promotes inflammation and immunosuppression (characterized by increased rates of cell division, DNA repair, oxidative stress and elevated inflammatory cytokines) and in turn causes cancer. Furthermore, it has been proposed that talc use during periods of ovulation may carry greater risk based on the hypothesis that ovarian surface epithelium disruption and repair accompanying ovulation might allow talc to become entrapped within inclusion cysts that form with ovulation. If true, we would expect to observe inflammatory reactions and fibrosis in “affected” ovarian tissue and inclusion cysts from talc users. However, of the limited studies, such as one by Heller et al.⁴⁸⁸ that evaluated tissue samples from talc users, ovarian tissue was not inflamed. Moreover, direct injection of talc into the ovarian bursa of animals has not been shown to promote inflammation or cancer.^{582,593}

Furthermore, it is unclear whether chronic inflammation is sufficient to induce OvCa in the absence of a carcinogen. If talc purportedly could cause cancer by promoting inflammation, we would observe increased cancers in patients undergoing pleurodesis—but that is not observed. Thus, even if signs of inflammation are detected in tissue, it does not mean that cancer will follow. Agents that should be protective against inflammation such as non-steroidal anti-inflammatory drugs (NSAIDs) have been shown in several studies to *not* protect against OvCa risk,^{410-412,419,539,652,419,420} which weighs *against* the inflammatory hypothesis, as discussed earlier in this report. Finally, a recent study found no significant correlation between serous carcinoma and histological signs of chronic tubal injury or inflammation.⁶⁵³ To argue in favor of the inflammatory hypothesis, Plaintiffs’ experts have recently cited work by Poole and colleagues,⁶⁵⁴ who measured the association of various inflammatory markers and OvCa development in the Nurse’s Health Studies 1 and 2 and the Women’s Health Study (WHS); the authors suggested

that C-reactive protein (CRP), a marker of global inflammation, is associated with a 53% increase in risk for OvCa for women with the highest (4th) quartile, compared with the lowest (1st) quartile of CRP levels in the blood. However, the relationship between perineal talc exposure and inflammation was not directly evaluated in this study. Moreover, the possibility remains that increases in CRP may be a consequence, rather than a cause, of the OvCa. Taken together, I do not find convincing scientific evidence for the inflammatory hypothesis as a mechanism by which talc could promote ovarian carcinogenesis.

From a molecular biology standpoint, mutations, or “spelling mistakes,” in critical genes and/or changes in the expression levels of genes or proteins involved in important biological pathways would be needed to cause cancer. Talc is typically inert and has *not* been shown to cause mutations or change levels of gene expression in ovarian cells.⁵⁷⁷ In fact, talc has been shown in some studies to have anti-cancer properties because it inhibits angiogenesis (the formation of blood cells that help feed the tumor)⁵⁷⁷ and can selectively promote apoptosis (cell death) of cancer cells, but not normal cells.^{578,655} The fact that talc is *not* mutagenic or genotoxic significantly discounts plaintiffs’ experts’ theories that talc causes OvCa.

Taken together, a convincing biological mechanism by which talc may specifically cause OvCa and other reproductive tract cancers is lacking. In attempting to identify causes of OvCa, it is important to consider alternate hypotheses that have biological plausibility and warrant evaluation, such as the incessant ovulation hypothesis,¹¹³ gonadotropin hypothesis,¹²⁷ and others related to endocrine disrupting chemicals.^{656,657}

6) Specificity (i.e., does a given exposure cause only one effect or disease or several diseases or outcomes?). According to Hill,⁶³⁹ there is an inherent relationship between specificity and strength in the sense that the more accurately defined the disease and exposure, the stronger the observed relationship should be. But the fact that one agent contributes to multiple diseases does not weigh against its role in any one disease. To evaluate the specificity of a potential talc-OvCa association, researchers can focus on whether existing data consistently indicate that perineal talc application is a) associated only with OvCa or with cancers in reproductive organs other than the ovary; and/or b) specific to certain histologic subtypes of OvCa. As mentioned earlier, similar to reports

focused on OvCa, associations have been reported between perineal talc exposure and endometrial cancer risk, albeit with inconsistent findings.⁶⁴⁵⁻⁶⁴⁸ With regard to the talc-OvCa associations being specific to a particular histology, reports that evaluated associations by histologic subtype have also had inconsistent findings, primarily regarding serous histology (**Table 4**).^{31,40,307,497,498,509,512,513,518,521,527,542} This lack of specificity is not surprising since OvCa is a heterogeneous disease with various histotypes having different risk factors.⁷⁻¹⁰ Taken together, the guideline of specificity is not met.

7) Coherence (i.e., does the interpretation of the data align with the history and biology of the disease?). Hill⁶³⁹ described coherence as “how well all the observations fit with the hypothesized model to form a coherent picture.” There appears to be a lack of a coherent biological mechanism for possible talc carcinogenicity and a lack of coherence to tie together findings from epidemiologic and molecular studies. For example, electron microscopy data is not coherent with an association between self-reported talc use and ovarian talc burden.⁵⁷⁹ Another factor that weighs against coherence is the fact that no studies have observed histopathological differences in the ovaries (or other reproductive organs) of talc users versus non-users.

Although some case-control studies^{337,467,518,521,556} and a meta-analysis²⁷⁶ reported a lower risk of OvCa among women who underwent pelvic surgery such as tubal ligation, particularly for serous and endometrioid OvCa (but not mucinous or clear cell) compared to women who did not undergo this procedure, other studies^{506,513,516} did not report a protective effect. Some suggest that surgically stopping the ascent of talc particles to the upper genital tract is an effective method to prevent OvCa risk by protecting the ovarian epithelium from exposure to agents such as talc, but it is important to consider that such surgeries are typically performed near the end of reproductive life when most talc exposure has already taken place. Moreover, these procedures can suppress ovulation. As will be described, the incessant ovulation hypothesis suggests that the lower the number of ovulatory cycles, the lower the risk for OvCa.¹¹³ It has also been suggested that tubal ligation can affect the uteroovarian circulation, leading to localized hypertension and damage to the ovary, resulting in reduced estrogen production.^{272,658} Additionally, Cramer⁵¹¹ proposed that there are uterine growth factors involved in ovarian carcinogenesis (such as insulin growth factor 1 and colony stimulating factor 1) that are

eliminated or reduced after pelvic surgery. Finally, it has recently been suggested that tubal ligation decreases growth capacity of the fimbriated epithelium, in turn supporting risk reduction of HGSOE.²⁸⁹ As such, by reducing levels of hormones and growth factors or inducing quiescence, tubal ligation may serve to protect against OvCa. Thus, alternate hypotheses that pertain to fewer ovulations and hormonal dysregulation (rather than physical blockage of potentially harmful agents) are available to explain how and why pelvic surgery may reduce OvCa risk.

8) Analogy (i.e., whether the theory resembles other accepted scientific relationships). Hill wrote that a causal claim can potentially emerge by comparing the evidence collected for one accepted causal relationship to that collected for another association not yet determined to be causal.⁶³⁹ Analogy involves comparing bodies of evidence in a systematic manner and assessing similarity (or lack thereof). In 1982, when Cramer et al.⁵⁰⁶ initially compared talc to asbestos, a known carcinogen, he suggested that use of talcum powder in the genital area was a potential risk factor for OvCa based in part on a “structural analogy” with asbestos. Although talc and asbestos are both magnesium silicates, they are structurally distinct and belong to different mineral groups and subgroups. Work by Stanton et al.⁶⁵⁹ shows that the carcinogenic ability/toxicity of fibrous asbestos is due to its structure. Moreover, talc is chemically inert. It has been argued that both minerals are similar in terms of accumulation and dissemination through lymph nodes in the body. For example, asbestos particle migration and entrapment in lymph nodes is correlated with asbestos burden in the lungs.⁶⁶⁰ However, self-reported talc exposure did *not* correlate with talc burden in the ovaries,⁵⁷⁹ and a study on talc burden in lymph nodes was based on a single case report of a woman with stage III serous OvCa who reportedly had a history of a tubal ligation and a 30-year history of perineal talc use.⁶²⁹ The talc-asbestos analogy is also not convincing because talc exposure is not associated with an increased risk of mesothelioma or lung cancer, cancers caused by asbestos. In short, analogy has not been established. Thus, the premise on which an analogy between talc and asbestos is made is flawed; this is guilt by association rather than scientific fact.

9) Experimental evidence (to support epidemiologic associations, investigators attempt to demonstrate that under controlled conditions, changing the exposure causes

a change in the outcome). No randomized trials exist regarding the purported perineal talcum powder-OvCa association, and it is unlikely that such data will become available in the future. Fortunately, as will be discussed, we can now draw from toxicological findings for experimental insight into causality, along with *in vitro* studies that test mechanistic pathways and demonstrate the biological role of an agent in disease initiation or progression. Such experimental studies do *not* support the talc-OvCa hypothesis.

B. Summary of Methodological, Statistical and Biological Concerns

In summary, application of the Bradford Hill considerations to the relevant published literature does not support a causal finding with respect to perineal talc exposure and the development of OvCa. Other factors discussed below further weigh against a causal conclusion:

- Imprecise exposure measurement is a paramount concern. Almost all epidemiologic studies that have examined a potential association between talc use and the development of ovarian cancer have relied on *indirect* measures of talc exposure ascertained via questionnaire (self-report or interviewer-administered). This is unavoidable because talc use cannot be verified in medical or pharmacy records, and quantification of a talc dose is challenging. Furthermore, body powders have a wide range of ingredients with varying talc content, including talc-free products. This makes it challenging to differentiate between different types of powder and their ingredients in epidemiologic studies. Self-report is a practical and inexpensive way to assess talc use, but it is prone to recall and interviewer bias.
- There has been extensive media coverage about the potential talc-OvCa association as a result of the ongoing litigation. As such, the potential for recall bias in case-control studies conducted since the litigation became public may be exacerbated, resulting in higher effect estimates/stronger reported associations. This concern has been noted and supported by several recent meta-analyses^{413,416} and other studies.^{527,531,661}
- Selection bias may have occurred in studies that had low or differential response or participation rates between cases and controls^{307,506,508,509,512,514,517,521,526,556,662} or non-responsiveness to questions

about talc.⁵¹⁶ Additionally, as compared to population-based studies, hospital-based studies^{136,510,516} are prone to selection bias because hospitalized patients (and control groups) are generally less healthy than the “general population” of cases and controls. However, there are several advantages to using hospitalized controls as well: (1) they may be more willing to participate in studies because of the convenience to do so; (2) they are more likely to be aware of prior exposures; and (3) they are more likely to be affected by the same intangible factors that influenced cases to present to a particular hospital. By contrast, population-based controls may be less motivated to participate in a study, and the quality of information they provide may differ from that provided by cases.^{508,525}

- There are multiple powder types that can be used by women (talc, baby, deodorizing, cornstarch or unknown), and each of these powder types varies in talc content. This could have led to exposure misclassification in the relevant literature.
- There are various possible routes of exposure to talc, including application directly on the perineal area or onto a sanitary napkin, a diaphragm, or a condom as a spray or powder or using swabs. The amount of talc applied or potentially contacting the ovaries, if any, may vary by mode of application.
- Assessment of self-reported talc usage differed across studies, with most reporting on ever/never use (**Table 1**), others reporting on frequency of use (times/week), and some reporting on duration (i.e., total years used, age/year at initiation and age/year at stoppage). The various ways talc exposure is measured make integration and interpretation very challenging (**Table 3**).
- Assessment of potential confounders of the talc-OvCa association differed across sites (**Table 1**). Such potential confounders include age, race/ethnicity, parity, history of tubal ligation, hysterectomy, oral contraceptive use, family history, douching, history of inflammatory conditions and agents (PID, endometriosis, and non-steroidal anti-inflammatory drugs). It is critical to assess the presence or absence of established and putative risk factors for OvCa among the study participants rather than only focusing on talc or a

possible link to asbestos. Year of birth and years of talc use are also important potential confounders that should be considered in studies of talc and OvCa risk.

Of note, cosmetic talc usage appears to be declining in the US. In 1988, Whittemore³³⁷ reported that up to 40% of US women used talc. Years later, the Nurse's Health Study reported that talc use (applied to the genital area) declined from 19% (for women born from 1920 to 1940) to 3% (for women born after 1975).⁴⁹⁷ Furthermore, the Historical Statistics for Mineral and Material Commodities in the US show that cosmetic talc use has substantially decreased in the US between 1982 and 2004.⁵³⁹ Although rates for new OvCa cases in the US have been falling an average by 1.5% each year over the last 10 years,⁶⁶³ if talc really was a causal agent that contributed to a substantial proportion of OvCa cases, we might expect that the dramatic decline in use would translate to a greater decline in OvCa incidence rates over the past few decades.

- In addition to difficulties of talc exposure assessment, OvCa is a challenging outcome to study because of its rarity and heterogeneity. Histologically, it can also be mistaken for pleural and peritoneal mesotheliomas, malignancies associated with asbestos use.
- Although some studies reported a stronger statistically significant association for the most common histologic subtype (serous),^{307,497,512,518,520,521,523,527,548} several case-control studies,^{509,513,525} a pooled analysis of case-control studies,⁵⁰⁴ three cohort studies,^{31,498,542} and a pooled analysis of prospective cohort studies⁵⁰⁰ did not confirm this finding (**Table 4**). Several studies reported an increased risk for endometrioid histology^{504,509,513,521}, but others did not.^{307,497,512,518,542} A statistically significant association restricted to clear cell histology was only found in the New England Case Control (NECC) Study as reported by Terry et al.⁵⁰⁴ However, three years later, Cramer et al.⁵²¹ also published on the NECC dataset, reported far fewer participants with clear cell histology and did not find an association. Of studies that detected a positive association with mucinous OvCa, none was statistically significant^{31,504,509,513,518,525} (**Table 4**).

IV. STATEMENTS ON TALC FROM NATIONAL AND INTERNATIONAL AGENCIES

Several national and international agencies and organizations study substances to determine whether they can cause cancer and/or promote cancer growth. These agencies evaluate potential carcinogenicity based on evidence from laboratory, animal, and human research studies. Below is a summary of the most recent position statements from some of the most prestigious health agencies, which are consistent with my opinions in this report.

In 2020, the **National Cancer Institute (NCI)** stated that “studies of women who used talcum powder (talc) dusted on the perineum have *not* found clear evidence of an increased risk of ovarian cancer” and that “the weight of evidence does not support an association between perineal talc exposure and an increased risk of ovarian cancer”²⁰² These statements were updated in 2024, and perineal talc exposure is listed under ‘Factors with *Inadequate* Evidence of an Association with the Risk of Ovarian, Fallopian Tube, and Primary Peritoneal Cancers” (<https://www.cancer.gov/types/ovarian/hp/ovarian-prevention-pdq>)⁵⁶⁶. The NCI Physician Data Query (PDQ) states “[r]esults from case-control and cohort studies are inconsistent, so the data are inadequate to support an association between perineal talc exposure and an increased risk of ovarian cancer.” They⁵⁶⁶ cite several meta-analyses^{414,500,504,550} and note that the ‘highly selected subset analysis from Woolen et al.⁵⁵⁰ that “should be interpreted with care.”

Consistent with the opinion formed in this report, Wentzensen, an NCI researcher, co-authored a review article⁶⁶⁴ that concluded the following: “[W]e currently do not understand the causal factors that underly the observed weak associations between genital powder use and ovarian cancer risk. . . .The low relative risk translates to a very low absolute risk increase, given the rarity of ovarian cancer. . . . Given the inability to attribute a clear causal factor to the observed associations, the lack of a good experimental model, the lack of a specific biomarker for powder-related carcinogenesis, and the inability to rule out confounding by indication, it is difficult to conclude that the observed associations are causal. Furthermore, given the widespread use of powders and the rarity of ovarian cancer, the case for public health relevance is limited.” In a

commentary on this article by Wentzensen⁶⁶⁴, Cramer⁶⁶⁵ cites methodologically flawed experimental studies^{608,631} in an attempt to support causality.

In 2022, the **American College of Obstetricians and Gynecologists (ACOG)**⁶⁶⁶ released responses to a list of frequently asked questions about OvCa. Listed risk factors for OvCa included age older than 55, family history of breast, ovarian, colon, or endometrial cancer, personal history of breast cancer, mutations in *BRCA1* and *BRCA2*, Lynch Syndrome, never having had children, infertility, and endometriosis. Similar to other organizations, talcum powder was not listed as an OvCa risk factor.

The **American Cancer Society (ACS)** currently includes information on “Talcum Powder and Ovarian Cancer” in an informational sheet⁶⁶⁷. The sheet states, “[f]or any individual woman, if there is an increased risk, the overall increase is likely to be very small. Still, talc is widely used in many products, so it is important to determine if the increased risk is real. Research in this area continues.” Talcum powder is currently listed on the ACS website under “factors with an unclear effect on ovarian cancer” rather than under the list of factors that increase risk of ovarian cancer which include getting older, being overweight or obese, nulliparity, taking unopposed ERT, having a family history of breast, ovarian, or colorectal cancer or a hereditary cancer syndrome (<https://www.cancer.org/cancer/types/ovarian-cancer/causes-risks-prevention/risk-factors.html>). Thus, talcum powder is not considered to be a factor associated with an increased risk for OvCa by ACS.⁵

In 2023, the **Center for Disease Control (CDC)**⁶⁶⁸ listed several risk factors for OvCa, including the following: being of middle age or older, having a family history of OvCa and/or a genetic predisposition to develop OvCa due to a *BRCA1/2* mutation or Lynch syndrome, having a personal history of breast, uterine, or colorectal cancer, having a history of endometriosis, having an Eastern European or Ashkenazi Jewish background, being nulliparous, and unopposed estrogen consumption for 10 or more years. Similarly, the **Society for Gynecologic Oncologists (SGO)**⁶⁶⁹ mentioned the aforementioned risk factors as well as a BMI of 30 or greater and also mentioned preventive factors including risk-reducing gynecologic surgeries, oral contraceptives, parity, and lactation. Genital talc use was not mentioned by the CDC or the SGO, again showcasing that national organizations do not endorse talc as a risk factor for OvCa.

The International Agency Research on Cancer (IARC)⁴⁶⁸ applies procedures for the scientific review and evaluation of potential carcinogens by independent experts and classifies agents into four categories: Group 1, carcinogenic to humans; Group 2A, probably carcinogenic to humans; Group 2B, possibly carcinogenic to humans; and Group 3, not classifiable. Based on limited evidence from human studies of a link to ovarian cancer, IARC has classified the perineal (genital) use of talc-based body powder as “possibly carcinogenic to humans (Group 2B).”^{468,670} This category is typically used for agents, mixtures and exposures for which there is limited evidence of carcinogenicity in humans and less than sufficient evidence of carcinogenicity in animals. Talc that contains asbestos is considered “carcinogenic to humans”; based on the lack of data from human studies and limited data in lab animal studies, IARC lists inhaled talc *not* containing asbestos as “not classifiable as to carcinogenicity in humans.” Finally, as summarized in 2021 by an expert panel from IARC and the NCI regarding the current state of knowledge and future priorities for OvCa research⁶⁷¹, “with the exception of oral contraceptive use, the field has not established modifiable risk factors for high-grade serous ovarian cancers.” Talc is not mentioned in this report.⁶⁷¹ Since the time of the IARC report, there have been additional prospective cohort studies, including the most recent by O’Brien and colleagues,⁵⁰⁰ that confirm the lack of a statistically significant association between perineal/genital talcum powder use and the lack of a dose-response relationship. There have also been case-control studies and meta-analyses that continue to observe a weak association. However, newer studies, such as one by Schildkraut and colleagues,⁵²⁷ suggest that recall bias played a role in positive associations.

To better understand the possible impact of recall bias on case-control studies of the perineal talc-OvCa relationship, a quantitative bias analysis was conducted by Goodman et al.⁶⁶¹ This analysis⁶⁶¹ used estimates of sensitivity and specificity data from the SIS study reported by O’Brien et al.⁵⁴³ and data from largest case-control study of OvCa and talc use by Cramer et al.⁵²¹ and showed through various scenarios that recall bias may significantly impact risk estimates, biasing them away from null.⁵²¹

The **US National Toxicology Program (NTP)** of the Department of Health and Human Services is formed from parts of several different government agencies, including the National Institutes of Health (NIH), the Centers for Disease Control and Prevention

(CDC) and the Food and Drug Administration (FDA). In 2016, the NTP did *not* include talc in its list of 248 agents known or anticipated to cause cancer in humans.⁶⁷⁴

A **Health Canada** assessment commissioned by the Minister of the Environment and the Minister of Health resulted in an evaluation of talc to determine whether it meets the criteria under paragraph 64c of the Canadian Environmental Protection Act (CEPA) and “may constitute a danger in Canada to human life or health.”⁵⁵⁵ According to paragraph 64 of CEPA,⁶⁷⁵ a substance is deemed to be toxic “if it is entering or may enter the environment in a quantity or concentration or under conditions that (a) have or may have an immediate or long-term harmful effect on the environment or its biological diversity; (b) constitute or may constitute a danger to the environment on which life depends; or (c) constitute or may constitute a danger in Canada to human life or health.” With regard to perineal exposure to talc, Health Canada concluded that “talc meets the criteria under paragraph 64(c) of CEPA as it is entering or may enter the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.”

The Health Canada assessment⁵⁵⁵ is the first government report that I have read that cited expert reports created for litigation rather than focusing on peer-reviewed literature; I am concerned that it does not provide a balanced view of the issues. When referring to human studies of the perineal-talc association, the Health Canada report mentions that meta-analyses^{418,435,448,414,502,504} <https://ocrahope.org/get-the-facts/faq/talcum-powder-and-ovarian-cancer/>, have “consistently reported a positive association” and then lists some component studies in a Table 6-1 that lacks details about the subgroups for which OR and 95% CI are reported along with adjustment factors. This is concerning because, as explained previously, apparent “consistency” of findings from meta-analyses (which include most of the same component studies) does not mean the findings of the component studies are valid. Moreover, as also mentioned previously, talc exposure data are based on self-report and measurement is imprecise. Finally, there was significant heterogeneity among the component studies, which should have precluded their integration.

Additionally, in the assessment of perineal talc, the Health Canada report⁵⁵⁵ cites several flawed studies in the subsection labeled “toxicokinetics.” The report discusses

talc particles being observed in the ovaries and/or lymph nodes of humans,^{488,579,629} but fails to mention the strong potential for contamination from surgical gloves or false positives due to a single radioactive tracer. The report⁵⁵⁵ also comments on migration of talc from the vagina to the ovaries in rats,⁵⁹⁷ and to movement of inert particles with the same size as talc as being transported from the vagina to the upper genital tract,⁶²³ pointing to retrograde menstruation as a possible mechanism. Yet, this discussion does not address studies finding that talc did not translocate to the ovaries after intravaginal injection of talc to rabbits⁶⁷⁶ or monkeys.⁶⁷⁷ Rather, the report mentions these studies^{676,677} elsewhere, stating that “rats are poor experimental models for perineal studies,” though the studies involved other animals as well. It should also be noted that in describing the experimental work conducted by Dr. Saed,⁶⁰⁸ the Health Canada report⁵⁵⁵ does not report on any of the numerous methodologic flaws noted by myself or others,⁶⁷⁸ and instead erroneously concludes that it “supports the hypothesis that talc exposure may lead to OvCa through inflammatory mechanisms.” Taken together, the conclusions drawn from the Health Canada report⁵⁵⁵ are unfounded; it is noteworthy that this report⁵⁵⁵ does not explicitly conclude that talc causes OvCa as plaintiffs’ experts suggest that it did.

The **American College of Obstetricians and Gynecologists (ACOG)** recently convened a panel of stakeholders and experts from many organizations (including ACOG, CDC, the American Academy of Family Physicians, and NCCN as well as patient advocacy groups including Facing our Risk of Cancer Empowered and the Foundation for Women’s Cancer) to summarize existing literature, best practices, and recommendations to guide developing evidence-based educational materials about OvCa. An executive summary from this conference by Burke et al.⁵⁶³ cited several meta-analyses^{413,414,416,679} on the purported association between talcum powder use and OvCa risk and concluded “the studies regarding the use of talcum powder and the risk of OvCa are heterogeneous”.

The **National Comprehensive Care Network** has published NCCN Guidelines version 1.2024⁹¹ which state that “[e]nvironmental factors have been investigated, such as talc, but so far they have not been conclusively associated with the development of this neoplasm [ovarian cancer].” Finally, the **Ovarian Cancer Research Alliance**

(OCRA)⁶⁸⁰ does not list talc as an OvCa risk factor and states that “[R]esearch regarding a connection between the use of talcum powder and increased ovarian cancer risk is inconclusive.”

V. CONSIDERATION OF PLAINTIFFS’ EXPERTS’ REPORTS

After reviewing the causation analyses set forth in reports of plaintiffs’ experts cited in the Health Canada assessment^{545,546,573,681-686} and in reports, amended reports, and supplemental reports of plaintiffs’ experts served in this proceeding, I have concluded that many of their opinions are “out of step” with the scientific community and unsupported by the relevant body of literature. Specifically:

Many of the plaintiffs’ experts describe cohort studies as being inferior to case-control studies in providing potential evidence for causality. This is contrary to the consensus of the scientific community, which recognizes that cohort studies are more reliable because they are less susceptible to various biases. No sound scientific justification is provided by plaintiffs’ experts for rejecting this well-established norm or hierarchy of scientific evidence. For example, Dr. McTiernan⁵⁴⁶ applies a hierarchy of her own which suggests that case-control studies are preferable when an exposure is difficult to measure and/or changes over time. This opinion is not accepted in the scientific community and appears to have been developed for litigation. Dr. Moorman⁵⁴⁵ states that she did not weigh one design (case-control or cohort) more heavily than the other, and that “all” studies provide evidence of causal effect. This is contrary to what I have learned as a trained epidemiologist—when assessing causality, it is critical to evaluate the strengths and weaknesses of individual studies, especially the biases and confounding factors that can be inherent to case-control studies. It is worth reiterating that case-control studies do not necessarily provide higher quality data. Although case-control studies may be easier to conduct, the temporal relationship between exposure and outcome is challenging to establish because data on the exposure are ascertained after the outcome. It is also difficult for a case-control study to investigate an exposure that changes over time; a cohort design is more likely than a case-control study to accurately investigate time-varying exposures. Thus, suggestions that case-control studies are superior are not supported by any strong rationale.

Plaintiffs' experts⁵⁴⁶ argue that cohort studies are limited because they study many outcomes and a wide variety of exposures in addition to talc and OvCa. This is in fact one of the many *advantages* of the prospective cohort design; inherent to prospective cohort studies is the advantage that they are designed specifically to evaluate numerous exposure-outcome relationships.

It is also argued that cohort studies are not able to accurately assess lifetime histories of talc exposure that would be needed to accurately estimate dose of exposure.^{545,546,573,683} For example, Dr. Smith-Bindman⁶⁸³ criticizes cohort studies for examining any talc use (which she refers to as "a weak, crude predictor"). But if Dr. Smith-Bindman believes the cohort studies suffer from assessing "any" use, then she should apply this criticism evenly to the case-control studies and meta-analyses that did the same. It is also worth noting that the questionnaires that case-control studies use to assess talc use habits are often not well designed or validated; yet, Dr. Smith-Bindman fails to note that. Imprecise exposure assessment is not unique to cohort studies, and it is not appropriate for plaintiffs' experts to criticize cohort studies on that basis, while ignoring similar issues in case-control studies. Plaintiff expert, Dr. McTiernan⁵⁴⁶, also erroneously concludes: "There were serious limitations to these cohort study analyses. None of the studies were specifically designed to investigate the relationship of talcum powder product use and risk of ovarian cancer. Rather, the cohorts were designed to study a large number of outcomes and a wide variety of exposures. Thus, none of the studies obtained detailed lifetime histories of talcum powder product use, although two did ask about duration of use for current users. None, therefore, was able to accurately measure dose of exposure. As explained in detail previously in this report, imprecise exposure assessment is an inherent flaw in every observational study that assesses self-reported exposures to an agent that is difficult to measure/quantify.

Plaintiffs' experts also criticize the relevant body of cohort studies for having supposedly small sample sizes. For example, Dr. McTiernan⁵⁴⁶ has criticized cohort studies that did not find an association between talc use and OvCa for including an insufficient number of cases to detect a statistically significant result. Essentially, Dr. McTiernan takes the position that because OvCa has a low incidence rate and few study participants developed the disease in the study and control populations, the studies were

not sufficiently powered to exclude the possibility of a relationship between talc use and OvCa. In evaluating this criticism, it is important to consider that the low overall OvCa incidence rate reported by Surveillance, Epidemiology, and End Results (SEER)¹² is estimated for *all* women; these rates differ by age and race/ethnicity and can change over time, with the rate of new cases reported as 10.9 per 100,000 women per year. The cohort studies^{31,497,498,542} represented in this report study a *subset* of the population and report incidences of OvCa that are actually *higher* than in the general population, potentially because they include women who may be at higher risk of developing OvCa due to factors such as family history or age. This means that the number of participants needed to detect a true risk is actually smaller. Further, as mentioned previously in this report, the meta-analysis by Berge et al.⁴¹³ estimated that the combined sample size of participants in the cohort studies had greater than 99% power to detect an OR of 1.25. Thus, the chance of erroneously concluding a lack of difference in OvCa risk between talc users and nonusers if there were a true increased risk with this exposure would be less than 1%. Additionally, the fact that very few women (<100/study) developed OvCa in these cohort studies regardless of their history of talc exposure underscores the small risk of developing OvCa in the high-risk populations that were studied. By no means does talc exposure increase OvCa risk to a degree that is statistically significant or clinically meaningful.

Cohort studies are also criticized by plaintiffs' experts for having insufficient follow-up periods that they claim do not consider the latency period for OvCa. For example, Dr. Siemiatycki^{572,684} erroneously reports that cohort studies follow people for two years, and Dr. Smith-Bindman states "several had very short follow-up periods."⁶⁸⁷ However, as described earlier in this report, the Gates study reported on women from NHS1 who had been followed for 24 years, the Women's Health Initiative Study followed women for 12.4 years (~>32.4 years of latency) and the Sister Study enrolled women between the ages of 35-74 and followed up after 6 years. Furthermore, O'Brien and colleagues⁵⁰⁰ extended follow-up by 20 years for NHSI, 5 years for WHI-OS, and 3 years for SIS.

It was perplexing that Dr. Smith-Bindman states in her expert report⁶⁸⁷ on page 30 that "this pooled data [by O'Brien et al.⁵⁰⁰] has limited usefulness in assessing the relationship between talcum powder use and ovarian cancer, in large part because it ignored most of the published literature," but then on page 36 praises the fact that the

Woolen et al.⁵⁵⁰ systematic review that she co-authored “included new and updated data from the Nurse’s Health Study cohort, showing greater risks among frequent daily talc users compared with less frequent users.” Thus, it seems contradictory that she criticized prospective cohort studies including NHSI for reportedly not having utility in evaluating a purported talcum powder-OvCa association, yet then included a highly selected subset of NHS data that she claimed to be useful in the context of their meta-analysis.

Plaintiffs’ expert Harlow⁶⁸⁸ also claims that “cohort studies can spuriously underreport true associations,” commenting that “findings from the Nurses’ Health Study are comparable with those observed in the multiple case-control studies. . . and that “the exposure assessment issues in the Women’s Health Initiative and the Sisters Study may explain why their results diverge from other studies.” As I mentioned previously, imprecise exposure assessment can affect study validity; it is an inherent flaw of every observational study that assesses self-reported exposures to an agent such as talc that is very difficult to measure and quantify. This is especially true for case-control studies in which recall bias related to exposure history is an issue. Additionally, Plaintiff’s expert, Dr. Cote⁶⁸⁹ suggests that a letter to the editor by O’Brien et al.⁶⁹⁰ (in response to one by Plaintiff experts Drs. Harlow and Rothman⁵⁶²) “reverses the conclusion of the original manuscript that stated there was not a statistically significant association between the use of powder in the genital area and ovarian cancer.” This is untrue; O’Brien et al.’s letter to the editor did not attempt to reverse their original conclusion regarding the lack of statistical significance between genital powder use and OvCa. An association was detected by O’Brien et al.⁵⁰⁰ among women with patent reproductive tracts (HR,1.13;95%CI,1.01-1.26). As mentioned earlier in this report, the effect size is small, and statistically significant associations were *not* found when restricting analyses to women with patent reproductive tracts among medically confirmed cases or when evaluating invasive status, tumor location, or histology. Thus, by no means is it correct to conclude that this large, well-powered, and rigorously conducted study⁵⁰⁰ with significant follow-up time supports a causal association between perineal talc use and OvCa risk.

Some plaintiffs’ experts ignored data that do not support the theory that talc use causes OvCa. Of particular concern are the unreliable meta-analyses conducted by plaintiffs’ expert, Dr. Smith-Bindman,⁶⁸³ in which she only included a restricted subset of

studies. For example, the subset of four studies that she relies on for an assessment of serous invasive OvCa is small, has substantially fewer cases than other meta-analyses, and selectively omitted the Gates study,³¹ which has 10 more years of follow-up than the Gertig study⁴⁹⁷ and would have conferred a lower RR. Furthermore, Dr. Smith-Bindman does not comment on the imprecision of self-reported talc use and invented her own definition of “regular” talc use as “ideally as daily or at least more than 3 uses per week.” Perhaps the most fatal flaw in the analysis is her identification of subgroups to be included in the meta-analysis *post-hoc*; this is contrary to the scientific method and frowned upon methodologically. Dr. Smith-Bindman also did not attempt to assess whether the studies she selected adequately controlled for confounding and bias before including them in her analysis. Finally, data from component studies were inaccurately abstracted and riddled with errors. For example, Dr. Smith-Bindman herself reported that she inaccurately abstracted data from the studies considered in her review.⁶⁸³ In particular, the confidence intervals for each of the 10 studies included in her Figures 2 and 3 do not match the information reported by the studies themselves. She⁶⁸³ also admitted that women with OvCa were likely counted twice in her analysis. Again, it cannot be underscored enough that accurate data abstraction and assessment of the quality of component studies is critical for conducting a sound meta-analysis. In summary, the meta-analyses conducted by Dr. Smith-Bindman⁶⁸³ are unreliable and faulty; they have countless errors and were designed to exclude data that do not support the hypothesis that talc use causes OvCa. It is concerning that plaintiff reports such as one by Dr. Levy⁶⁹¹ do not list any limitations of the meta-analysis by Woolen et al.⁵⁵⁰

In a non-peer-reviewed meta-analysis, Dr. Siemiatycki,⁶⁸⁴ in his expert report dated November 16, 2018, calculated the RR between perineal talc exposure and the development of ovarian cancer to be 1.28 (95% CI: 1.19-1.38). (I note that in his amended report, he calculated the RR as 1.30 (95% CI: 1.21, 1.40).) The analysis appears to have been conducted properly, but his conclusion that a RR of 1.28 (or 1.30) is “high” is surprising because it is widely understood in the scientific community that such an RR is weak and therefore could result from bias, confounding and/or random error rather than a true causal relationship. It is also concerning that Dr. Siemiatycki⁶⁸⁴ states that “the statistical significance of individual studies is irrelevant to the consideration of causality;

it is the totality of evidence embodied in the meta-analysis that counts.” When evaluating the purported talc-OvCa association, we cannot ignore the design and findings of individual studies by combining them, particularly when the individual studies were heterogeneous in their design and analysis. Additionally, statistical significance cannot simply be ignored. Dr. Siemiatycki⁶⁸⁴ erroneously states that the statistical significance of component studies is irrelevant to the consideration of causality and claims that the totality of evidence encompassed by meta-analyses is most relevant. As I have expressed previously, it is essential to consider the quality of the component studies as well as their design when interpreting findings from a meta-analysis.

Misconceptions are also present in the plaintiffs’ epidemiologists’ applications of the Bradford Hill framework as it applies to three main criteria: strength of association, consistency of association and dose-response. It is important to reiterate the following points that are discussed in prior sections of this report:

Weaker strengths of association (such as those detected by some of the case-control studies) are more likely to be spurious (due to confounding and biases in the study design). Plaintiffs’ experts^{545,573,683,684,689} seem to mischaracterize the weak association between talc use and OvCA as one that is “strong” or one that “could *not* have occurred by chance.” Plaintiffs’ experts⁶⁸⁸ also misleadingly conclude that “the persistent association between talc use and ovarian cancer is not readily explained by any non-causal hypothesis. . . and that causation is the most reasonable explanation for the association between perineal exposure to talc and ovarian cancer.” Plaintiffs’ experts have claimed that the associations are “strong” because of the public health impact. For example, Dr. Smith-Bindman⁶⁸³ concludes (without any pertinent citations) that because a large number of OvCa cases are purportedly caused by talcum powder, and talcum powder use provides no medical benefit, strength of association is important and met. However, this is misleading and circular because there would only be a public health impact if the association were causal. Importantly, disease prevalence or severity does not impact the strength of an association. Rather, as stated by Dr. Wentzensen,⁶⁶⁴ “[g]iven the rarity of ovarian cancer in the general population, the small increase in relative risk translates to a very low increase in absolute risk.”

Plaintiffs' experts^{545,546,573,689} assert that the consistency criterion is satisfied despite the inconsistent findings cited previously in this report. As an example, Dr. McTiernan⁵⁴⁶ states that "the association between use of talcum powder products and risk of ovarian cancer was highly consistent," Dr. Singh⁵⁷³ concludes that "the direction and strength of association of talc and ovarian cancer is generally consistent across studies," and Drs. Cote⁶⁸⁹ and McTiernan⁵⁴⁶ partly base their opinion on causality from "the consistency of results across geographic areas and in different racial/ethnic groups." Although there are some consistencies among the studies, they are among hospital-based case-control studies and cohort studies, which do *not* reveal statistically significant associations between talc use and OvCa risk. On the other hand, inconsistencies exist between hospital-based and population-based case-control studies and within population-based case-control studies. This lack of consistency within and between study designs, time and location suggests that any association may result from bias, confounding and/or random error, and therefore weighs against a causal relationship.

Plaintiffs' experts^{546,573,683} also claim that there is a dose-response relationship where no clear patterns are demonstrated. Dr. Siemiatycki⁶⁸⁴ states that "there is a clear indication of increasing risk with increasing cumulative exposure," and cites studies by Terry et al.⁵⁰⁴ and Schildkraut et al.⁵²⁷ in his early reports and adds in Woolen et al.⁵⁵⁰ in his amended report.⁵⁷² Siemiatycki⁵⁷² states that "the most convincing of these is the Terry 2013 pooled analysis, which assembled a larger dataset than all other attempts to assess dose-response combined." However, Terry et al.⁵⁰⁴ concluded that "among genital powder users, we observed no significant trend ($P=0.17$) in risk with increasing number of lifetime applications." Thus, a significant dose response was *not* observed by Terry et al.⁵⁰⁴ Only a few case-control studies^{509,512,513,518,521,525,527} have purported to detect a dose-response relationship and none of the cohort studies has found one. Notably, among the numerous case-control studies that have not reported a dose-response relationship are several studies that did analyze "cumulative" talc use as defined by frequency and duration. As an example, Mills et al.⁵¹⁸ evaluated cumulative dose by quartiles and reported a trend that does not support a dose response. Cook and colleagues⁵¹² also looked for an association across strata of "cumulative lifetime days" but did not identify an increased risk for any of the strata/categories, with relative risks for

the lowest and highest strata being similar. Rosenblatt et al.⁵²⁵ and Chang et al.⁵¹³ also evaluated the association across categories of increasing lifetime applications and reported the lowest (and even inverse) associations for higher use categories. Experts Dr. McTiernan⁵⁴⁶ and Moorman⁵⁴⁵ also refer to the analysis by Woolen et al.⁵⁵⁰, with both claiming that the higher reported pooled OR for frequent talc use in the Woolen meta-analysis⁵⁵⁰ as compared to the OR reported in prior meta-analyses reporting on ever use of talc supports a dose-response relationship between genital talc use and ovarian cancer. It is scientifically unsound to make this conclusion because Woolen et al.⁵⁵⁰ do not attempt to measure or address dose response. Moreover, their study is methodologically flawed as mentioned previously in the current report. It has also been argued by some plaintiffs' experts that this lack of a consistent dose-response relationship could be explained by a threshold effect⁶⁸⁷. However, if that were true, one would expect to observe consistent findings for that supposed threshold, as well as biological data to support the relationship; none exist.

When offering mechanisms by which talc could cause OvCa, plaintiffs' experts^{545,546,573,683,687} contend that talc or other constituents can travel from the perineal area up the genital tract (against gravity and menstrual fluid) to the ovaries. Plaintiff's experts⁶⁸⁹ admit that "animal studies do not provide consistent evidence of translocation of talc." They^{545,546,573,683} also propose inhalation and the lymphatic system as mechanisms for talc-based ovarian carcinogenesis. Furthermore, several plaintiffs' experts^{545,546,573,683,684,687,689,691} assert that talc causes inflammation and promotes OvCa, despite the lack of scientific support. In support of these claims, they cite methodologically unsound laboratory studies^{606,608,615}. For example, Plaintiffs expert, Dr. Levy,⁶⁹¹ cites work by Saed and team⁶⁰⁶ as evidence to support the hypothesis that talc promotes OvCa through chronic inflammation, but he fails to acknowledge any flaws in their⁶⁰⁶ methodology. In the conclusion of Levy's report,⁶⁹¹ he states "Talcum powder product-induced inflammation causes damage to the DNA, genetic mutation, genomic instability, and cell transformation." He⁶⁹¹ also states: "The properties and constituents of talcum powder products act as inflammatory agents and the role of inflammation in triggering oxidative stress, activating cytokines, cell proliferation, DNA damage, and genetic mutations (such as SNVs) provide a biologically plausible mechanism for the

carcinogenicity of talcum powder products.” Furthermore, in his report⁶⁹¹, Dr. Levy erroneously comments that “chemical and other environmental agents, such as talcum powder products, can cause mutations that, acting along with inherited mutations, cause ovarian cancer.” These statements are misleading and untrue and lack support of sound scientific studies demonstrating genotoxicity of talc.

To attempt to provide support for his hypothesis that talc induces inflammation and subsequent OvCa, Dr. Levy⁶⁹¹ also reports on a study by Brieger et al.⁶⁹² that used pooled data from 8,147 women with invasive EOC cases from OCAC to evaluate the association between pre-diagnosis inflammatory-related exposures (which included alcohol use, aspirin use, other non-steroidal anti-inflammatory drug use, body mass index, environmental tobacco smoke exposure, history of pelvic inflammatory disease, polycystic ovarian syndrome, endometriosis, menopausal hormone therapy use, physical inactivity, smoking status, and talc use) and overall survival. The authors⁶⁹² created an inflammation-related risk score (IRRS) and reported that women in the upper quartile of the IRRS had a 31% higher OvCa death rate than women in the lowest quartile (95% CI 1.11–1.54). Although the findings are suggestive of a potential role for a high inflammation score as a marker of poor prognosis, it should be noted that the authors did not adjust for debulking status, which is an important prognostic factor. Additionally, data on talc use was missing for 45.7% of the women in the study. Of women for whom talc use status was available (n=3,725), no association was identified for those who reported genital use (HR 0.94, 95% CI: 0.84-1.04) or use on non-genital area (HR, 95% CI: 0.84-1.08). Thus, it remains unfounded for Dr. Levy⁶⁹¹ to conclude, “inflammation from any source is potentially harmful, and when inflammation is introduced chronically to the genital region using external compounds such as talcum powder, the risk of developing ovarian cancer increases.”

Collectively, the evidence cited by Plaintiffs for proposed mechanisms by which talc may cause OvCa is weak and speculative, sentiments echoed by IARC⁴⁴⁹ and numerous authors mentioned herein, including those who published recent meta-analyses.^{413,416} Moreover, despite consistent messages from professional organizations that inconclusive evidence exists to support talc as a risk factor for OvCa, it is also

concerning that plaintiffs' experts do not comment on them at all or explicitly state that they do not "put much weight onto these statements" as stated by Dr. Cote ⁶⁸⁹.

Finally, I note that it does not seem that much weight is put into the importance of familial or hereditary OvCa by Plaintiff's experts. For example, although Dr. Levy⁶⁹¹ acknowledges that "[G]enetic testing is a powerful and valuable tool to guide cancer treatment, prognosis, and family planning, his report does not explicitly mention that significant risk factors for OvCa include having a family history of breast and/or ovarian cancer or the presence of a germline mutation in *BRCA1/2* or other genes such as *MUTYH* as mentioned by ACS⁶⁹³. He⁶⁹¹ also fails to comment on the utility of sequencing of tumor tissue as a complement to germline testing. With respect to Dr. Levy's discussion of specific Plaintiffs, below is a summary of my observations based on my experience as a trained genetic counselor.

With respect to Ms. Bondurant, Dr. Levy omitted pertinent information related to the *SDHA* mutation that was detected in her germline and details of her family history of cancer. For example, available records show that the *SDHA* mutation identified in Ms. Bondurant was reclassified from 'likely pathogenic' to 'pathogenic'. BondurantL-AGC-00024-25; BondurantL-AGC-00033-34. Although germline *SDH* mutations (including those in *SDHA*) are typically associated with pheochromocytomas, paragangliomas, and gastrointestinal stromal tumors⁶⁹⁴, a germline *SDHA* mutation has also been reported in a woman who also had clear cell ovarian cancer and a history of endometriosis⁶⁹⁵, similar to Ms. Bondurant. Moreover, *SDHA* overexpression and amplification has been implicated in ovarian tumorigenesis.^{696,697} Thus, there is biological plausibility for how a pathogenic germline *SDHA* mutation could contribute to OvCa susceptibility. Additionally, emerging data support a rise in the detection of pathogenic *SDHA* mutations as secondary findings when conducting multi-gene panel testing in patients without a personal or family history of one of the typical *SDHA*-associated tumors (Skefos, 2024)⁷⁰². Ms. Bondurant's testing also reported a VUS in the *PTCH1* gene. BondurantL-AGC-00024-25. Medical records also include references to multiple relatives with cancer, some of whom were not mentioned by Dr. Levy. Although there are some inconsistencies in the records and materials that I have reviewed for Ms. Bondurant, these records and materials refer to the following family history: a maternal aunt with ovarian cancer around

age 81; another maternal aunt with breast cancer in her late 50s; a maternal uncle (or first cousin) with pancreatic cancer in his mid 60s; a maternal uncle with colon cancer at age 75; a mother with an ovarian tumor (reportedly benign) around age 61; a brother with non Hodgkin's lymphoma; and a maternal grandmother with lymphoma. BondurantL-AGC-00041; BondurantL-TCCMR 01627-1631, 1632-35; Plaintiff Profile Forms. And there is also a mention that Ms. Bondurant's mother was diagnosed with breast cancer in 2020. Plaintiff Profile Forms. Based upon my experience in genetic counseling, Ms. Bondurant's family history strongly suggests the possibility of hereditary cancer risk and hereditary breast and ovarian cancer syndrome (HBOC) in particular. This is also noted in her records. Despite her germline genetic testing results to date, her family history cannot be dismissed. Unfortunately, next generation sequencing of her tumor tissue was unable to be successfully performed by Caris Life Sciences because of insufficient quantity and/or quality of DNA and RNA. BondurantL-CLS-00005-9. Taken together, the possibility remains that Ms. Bondurant's OvCa is linked to her *SDHA* germline mutation, her history of endometriosis, and/or a yet-to-be-identified mutation causing HBOC.

With regard to Ms. Converse, an Ashkenazi Jewish woman diagnosed with clear cell OvCa at age 58, Dr. Levy does mention a significant family history of cancer on her maternal side of the family. However, he does not provide specifics beyond Ms. Converse's mother, who was diagnosed with breast cancer at age 46. It is noteworthy that her maternal grandmother had pancreatic cancer in her 80s, and that she had a paternal aunt and cousin diagnosed with breast cancer in their 70s and 40s, respectively. CONVERSE_HILARY_GENETICSTESTING_00007; ConverseH-YUGOCMR-00096-98. Records also refer to a maternal uncle with non Hodgkin's lymphoma. CONVERSE_HILARY_GENETICSTESTING_00007; ConverseH-YUGOCMR-00096-98. Ms. Converse tested negative for the three founder mutations in *BRCA1* and *BRCA2*, commonly identified in Ashkenazi Jewish populations, and subsequently tested negative for other *BRCA1* and *BRCA2* mutations via Comprehensive BRCAAnalysis testing through Myriad Genetics. ConverseH-YUGOCMR-00099; ConverseH-YUGOCMR-00101. As mentioned by Dr. Levy, additional testing performed at Yale identified variants of unknown significance in *ATM* (L2307F) and *TGFBR2* (A329T). ConverseH-PHPMR-

00414. Both of these variants were later identified in her mother who had early-onset breast cancer, but to date, ClinVar suggests the pathogenicity of these variants is unclear. CONVERSE_HILARY_DRPETERSCHWARTZ_00008. Nevertheless, mutations in *ATM*, *BRCA1/2*, and other homologous recombination genes have been identified among women with non-serous histology, including clear cell subtypes and early-onset breast cancer. In 2017, Ms. Converse was informed that “testing options for hereditary breast and ovarian cancer really have not changed substantially since she had her panel testing in 2013-2014.” ConverseH-YCGCMR-43-44. Based on Ms. Converse’s significant personal history of ovarian cancer and family history of early-onset breast cancer and pancreatic cancer, it is my opinion that this is a high-risk family that should adhere to management recommendations of HBOC families. My recommendation aligns with those from the genetics team at Yale University.

Ms. Judkins was diagnosed with high grade serous ovarian cancer at age 60 and had a family history of breast cancer in a paternal great-aunt at an unknown age. JudkinsC-AGMR-00019. Additional records state or indicate that this paternal aunt may have been diagnosed in her 60s, and that Ms. Judkins may also have another paternal aunt who was deceased from breast cancer. JudkinsC-DHMCMR-632. Deposition testimony also revealed a grandmother with pancreatic cancer; a paternal first cousin with prostate cancer; and a grandfather or maternal uncle, each having bladder or kidney cancer. May 11, 2021 Deposition Transcript of Katherine Downs, p. 41; December 1, 2020 Deposition Transcript of Anne Carter Judkins, p. 91-92. Ms. Judkins was found to have a variant of uncertain significance in the *PTEN* gene known as c.-1283G>A as part of the Ambry Genetics OvaNext panel, which tests for alterations in 25 genes. JudkinsC-AGMR_00006-9. Tumor testing at Myriad Genetics was negative for *BRCA1* and *BRCA2* mutations and was positive for genomic instability associated with homologous recombination deficiency, suggesting she may have been a candidate for treatment with a PARP inhibitor. JudkinsC-MGMR-00003-4. Germline *PTEN* mutations are associated with Cowden Syndrome which is characterized by benign and cancerous tumors of the breast, thyroid, uterus, colorectum, kidney, and skin. Despite early reports that *PTEN* mutations are not features of OvCa,⁶⁹⁸ *PTEN* is involved in homologous DNA repair and has been shown to be altered in the germline (particularly in the 5’ and 3’ untranslated

regions) in a study of patients with breast cancer and OvCa^{699,700}. The c.-1283G>A variant detected in the blood of Ms. Judkins is located in the 5' untranslated region of *PTEN* in the promoter region. Although its clinical significance is unknown at this time according to ClinVar, variants in this region have been shown to be deleterious and to produce reduced PTEN protein levels⁷⁰¹. Thus, based on her personal history of OvCa, family history of breast cancer, and *PTEN* variant in the promoter region of the gene, this family should be followed closely with cancer surveillance.

With regard to Ms. Newsome, a woman diagnosed with endometrioid adenocarcinoma of the ovary at age 53, germline testing performed at Myriad Genetics revealed a variant of uncertain significance known as c.1513T>G (p.Cys505Gly) in the *MUTYH* gene. NewsomeT-MGIMR-3. Although the clinical significance of this variant is unclear, and the genetic testing report indicates “there are currently insufficient data to determine if these variants cause increased cancer risk,” *MUTYH* mutations have been observed in the germline of women with OvCa. Dr. Levy does not present any information about the fact that *MUTYH* mutations have been associated with OvCa susceptibility. Like several other plaintiffs, Ms. Newsome has an extensive family history of cancer, including renal cell cancer in her father, pheochromocytoma in a brother, prostate cancer in a maternal uncle, and a paternal first cousin with salivary gland cancer and, 20 years later, prostate cancer. NewsomeT-HCHMR-130-132; NewsomeT-MGIMR-3-6; December 9, 2020 Deposition Transcript of Tamara Newsome, p. 122-123. Additionally, Ms. Newsome reported that her maternal grandmother had a sister who had been diagnosed with ovarian cancer and lived to 106 years old. December 9, 2020 Deposition Transcript of Tamara Newsome, pp. 124-125. Noteworthy is that *MUTYH* mutations have been reported in individuals with renal cell cancer (Zeng, 2022; Ouedraogo, 2023).^{703,704} Additionally, renal cell cancer, ovarian cancers of various subtypes, and pheochromocytomas are also found in families with Von-Hippel Lindau Syndrome who harbor mutations in the *VHL* gene (Papageorgiou, 2002).⁷⁰⁵ The *VHL* gene is not part of the multigene panel that was used by Myriad Genetics to evaluate the germline of Ms. Newsome. Taken together, the possibility remains that there is a familial or inherited susceptibility to the cancers observed in Ms. Newsome and her family members.

VI. SUMMARY AND FINAL CONCLUSIONS

Based on the accumulated data discussed in this report, there continue to be *established* risk and protective factors for OvCa (i.e., family history, parity, oral contraceptive use) as well as factors that may possibly confer OvCa risk or protection. There are also other factors that have been evaluated and shown to have inconclusive or inconsistent weak effects that are unlikely to be causal (**Table 6**). Due to failure of multiple types of studies to show a consistent and coherent biologically plausible relationship between talc and OvCa, a proper scientific analysis leads to the conclusion that there is *not* a causal relationship between talc and OvCa. The puzzle pieces I have examined and attempted to put together from various disciplines do *not* create a cohesive picture to support talc as a contributor to ovarian carcinogenesis. Simply put, *causation cannot be proven* based on data provided to date. Key methodological, statistical and biological concerns leading to this conclusion include: the imprecision related to self-reported measures of talc; selection bias; recall bias; exposure and disease misclassification; absence of strong associations; inconsistency of weak associations between and across study designs; lack of adjustment for important confounders in some studies; lack of demonstrable dose-response relationships; the faulty comparison/analogy between talc and asbestos along with a lack of data that strongly support asbestos as a risk factor for OvCa; failure to demonstrate a coherent and biologically plausible mechanism explaining how talc could migrate to the ovaries and cause OvCa and not cause cancer of other organs along the reproductive tract; failure to consider hypotheses other than the inflammatory hypothesis; lack of evidence from clinical research and laboratory studies of humans; lack of supporting data of talc carcinogenicity from *in vitro* studies; and lack of supporting evidence of talc carcinogenicity from *in vivo* studies.

Next, I have included a “score card” that I used to assess the purported talc-OvCa association. This score card incorporates recognized methodology and factors used in the field of epidemiology to assess whether an association is causal.

Factors to Consider When Assessing the Reported Talc-OvCa Association	My assessment
Could the association be spurious due to chance or bias?	Yes
Could the association be non-causal due to confounding or because the outcome preceded the exposure?	Yes
Is the association causal ?	No
Did the exposure precede the outcome?	Usually
Is the association strong?	No
Is the association consistent within and across designs and populations?	No
Is there a clear dose-response relationship?	No
Is there biological plausibility?	No
Is there compelling experimental evidence?	No

As a female cancer researcher, patient advocate, former genetic counselor, and woman who has lost numerous friends and patients to OvCa, I want nothing more than to identify exposures that can be modified or removed from the lives of women at increased risk to develop this dreadful disease. In my expert opinion, the amount of time, money and energy that has been taken to “blame” talcum powder for causing OvCa needs to be placed elsewhere, as there are many more likely reasons (some known and some unknown/understudied) to explain why OvCa may have occurred. All of my opinions in this report have been set forth with a reasonable degree of scientific certainty. I reserve the right to amend or supplement this report as new information becomes available and/or is provided to me, and to review and comment on other legal expert reports.



Signed by Jennifer B. Permuth

Date: 5/28/2024

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